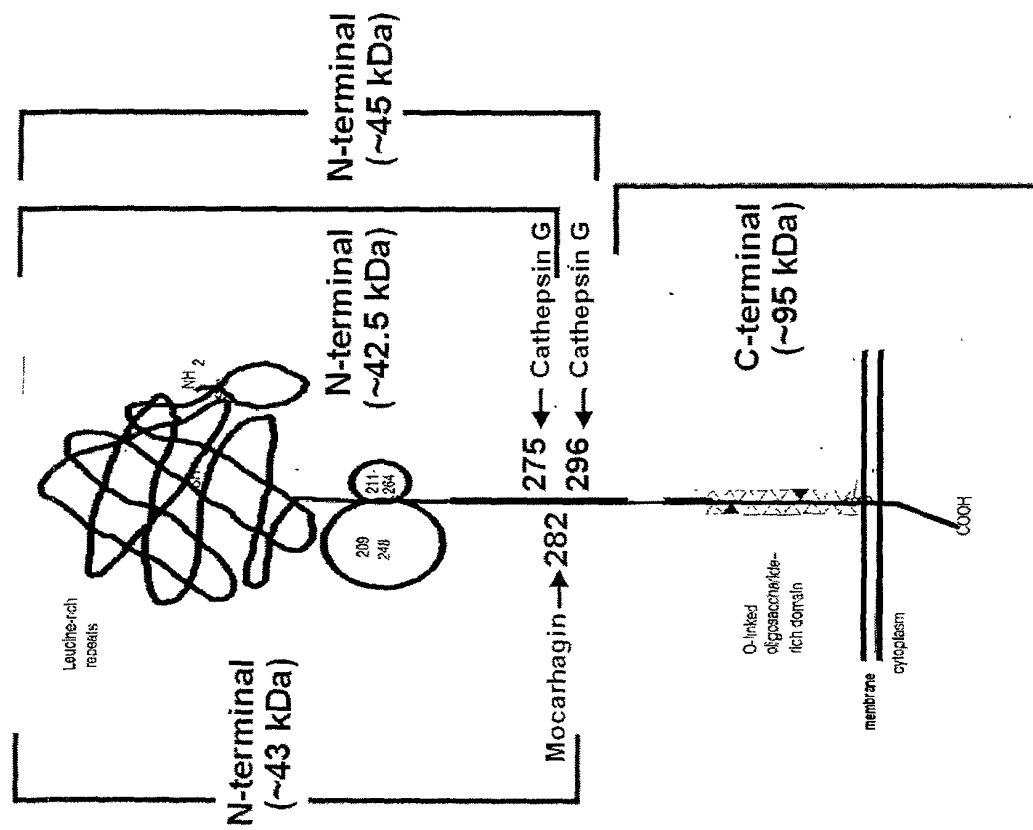


**FIG. 1**

# **Cleavage sites of endoproteases on the a-chain of GPIb**



# Binding of Y1 and Y17 to platelets in reduced and non-reduced conditions

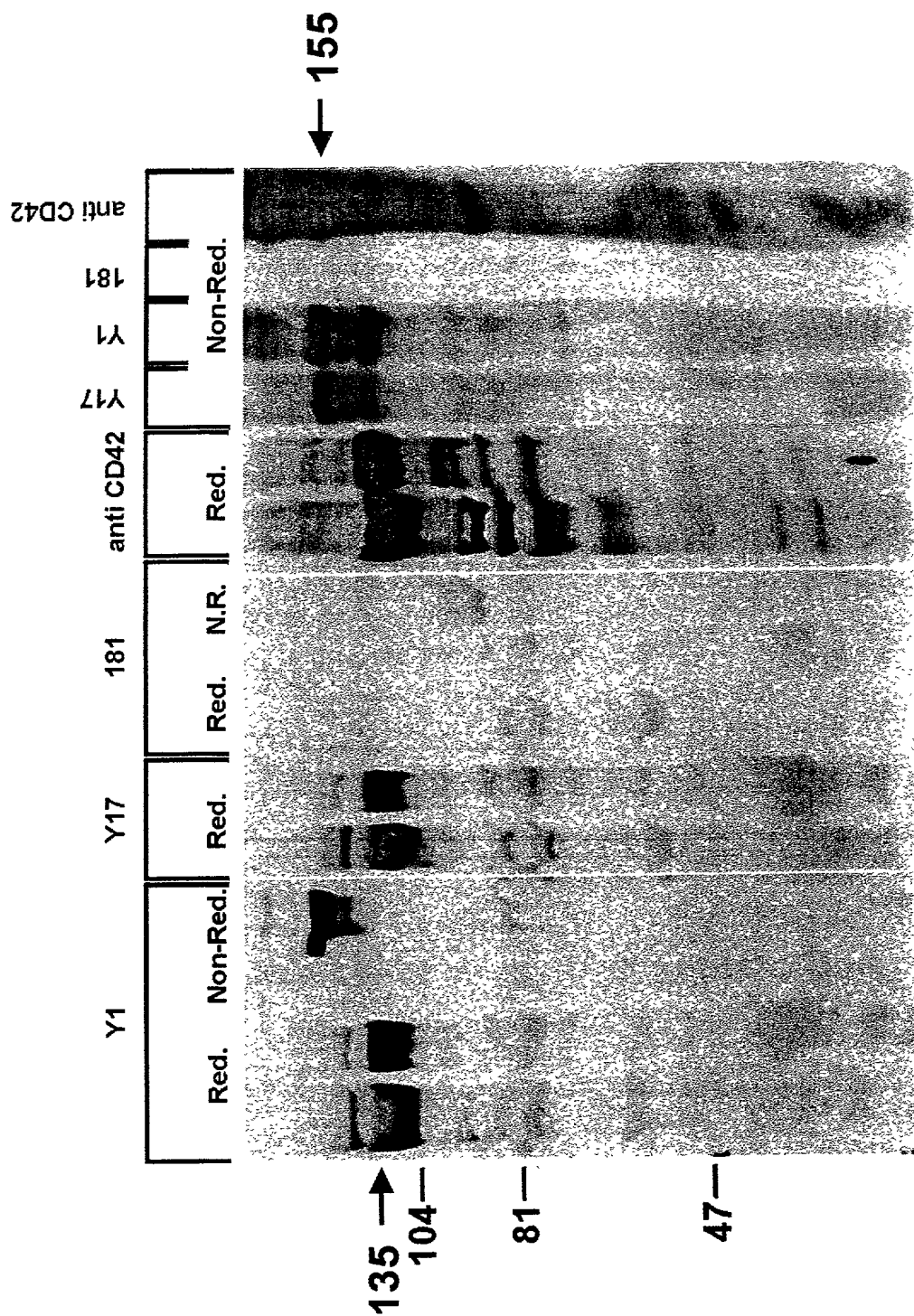


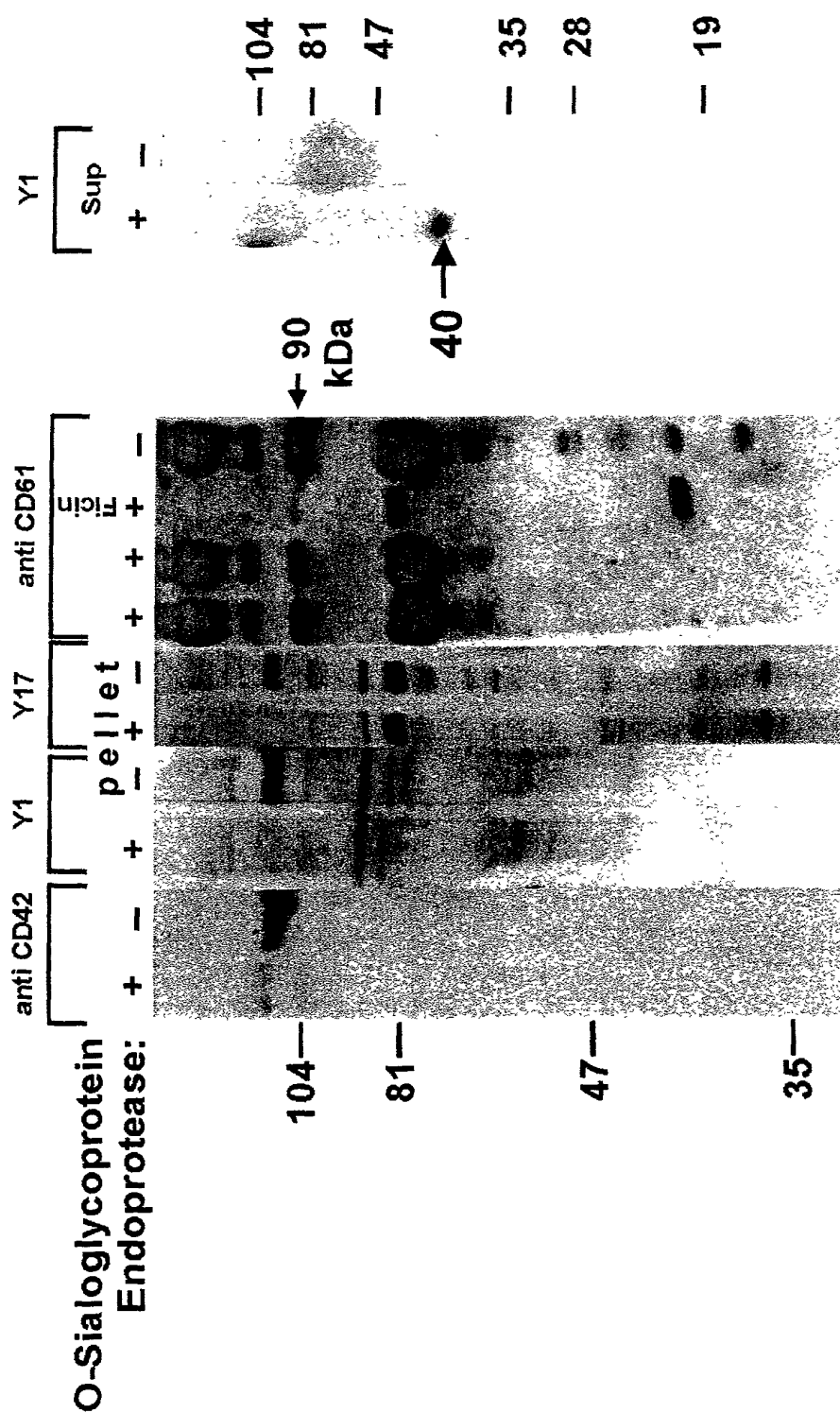
FIG. 2

# Characterization of Optimal Determinants for Binding of Y1 to It's Ligands

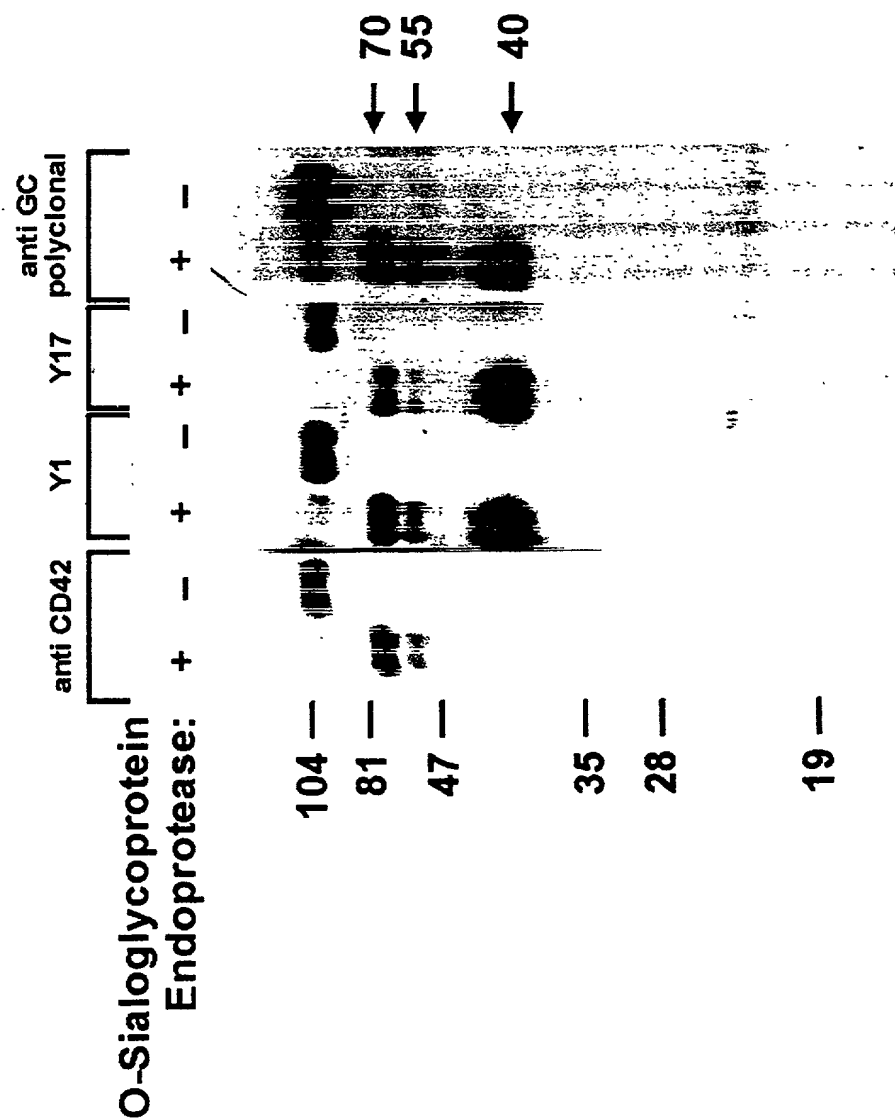
**FIG. 3**

	Platelets/GC	KG1/RP-HPLC #4
Rec: GP1b 1-340 GP1b 1-480	- -	
Glycanase: N N+O	+ +	+++ +++
Proteases: Mocarhagin O-Sialo Peptidase Ficin Trypsine Elastase	++ (~40kD) ++ (~40kD) - ++ (~40kD) ++ (~40kD)	- - - - ++
Sulfatase (Aryl)		-/+

# Cleavage of platelets GPIb by O-Sialoglycoprotein abolishes binding of both Y1 and Y17



Y1 and Y17 binds similar glycosialin fragments after cleavage by O-Sialoglycoprotein Endoprotease



# Specific GPIb Proteolysis Abolishes Y1 Binding to Platelets

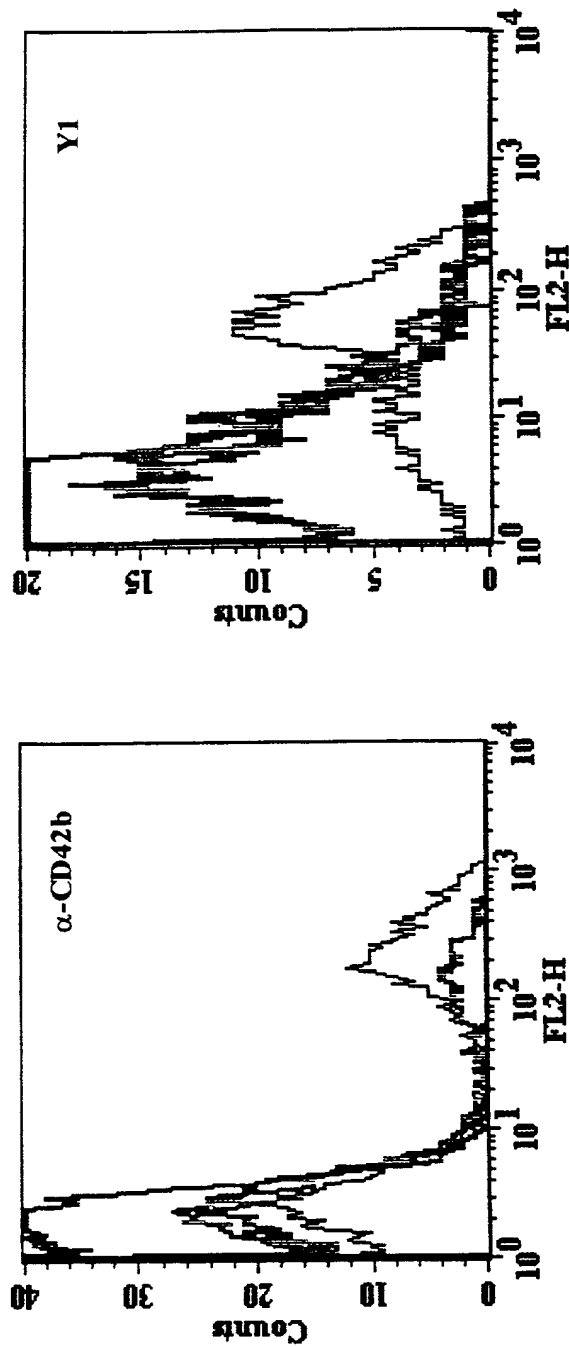


FIG. 6

Key	Name	Parameter	G
	NON-TREATED PLATELETS		
—	O-SIALOGLYCOPROTEIN ENDO. (10 μg/ml)		
—	O-SIALOGLYCOPROTEIN ENDO. (50 μg/ml)		
—	FICIN (18 μg/ml)		

Western blot analysis of mocarhagin expression in *E. coli* strains. The blot shows bands for various strains, with molecular weight markers at 104, 81, 47, 35, and 28 kDa. A prominent band is labeled ~40 kDa. The lanes are grouped by anti-CD42 C-terminal (CG, +, -) and anti-CD42 N-terminal (CG, +, +, +, +).

# Binding of Y1 and Y17 to glycolalycin after cleavage by mocarhagin

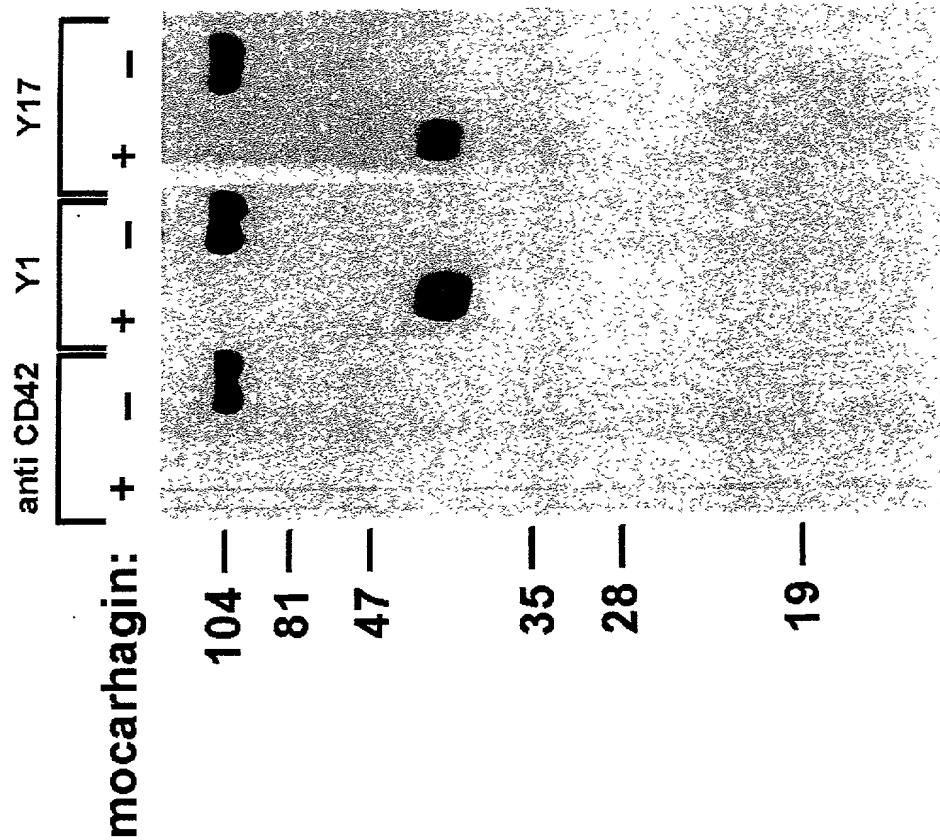
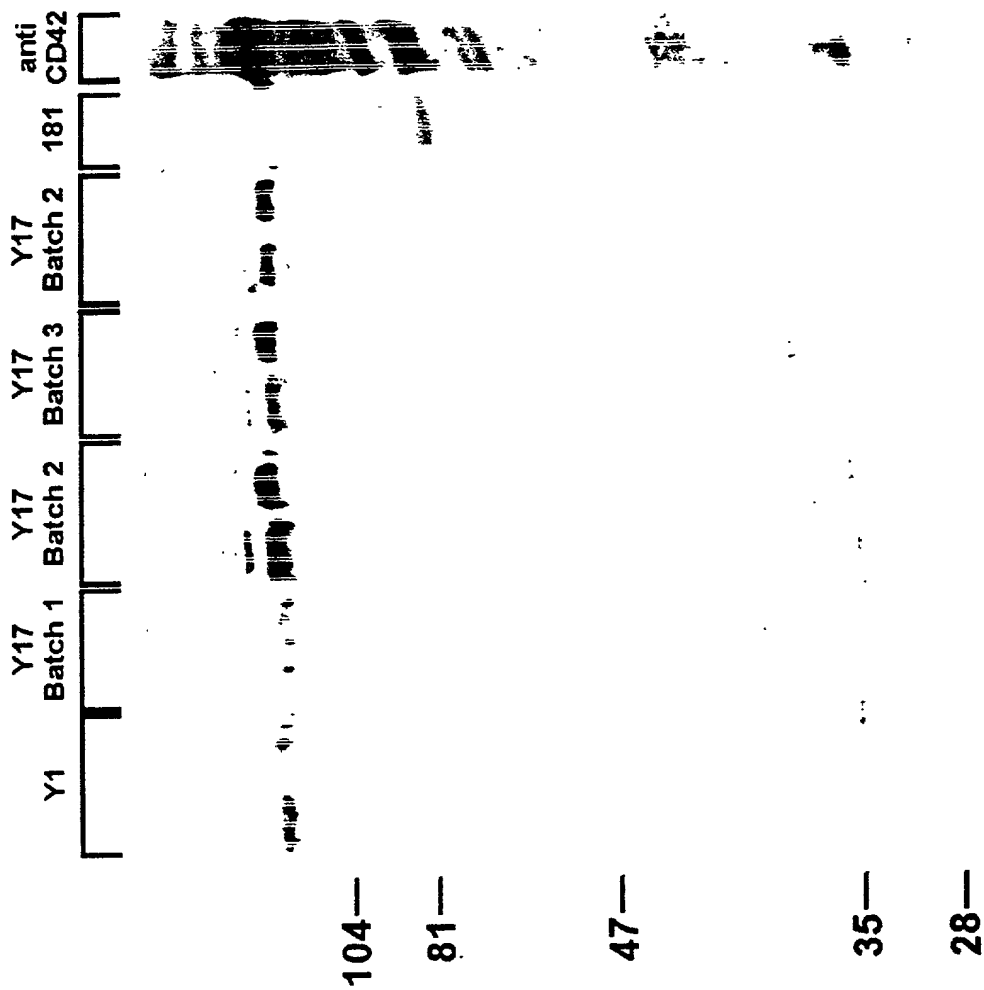


FIG. 8



FIG. 9

# Binding of Y1 and Y17 to platelets



Y1 and Y17 bind glyocalycin similar after cleavage by Ficin

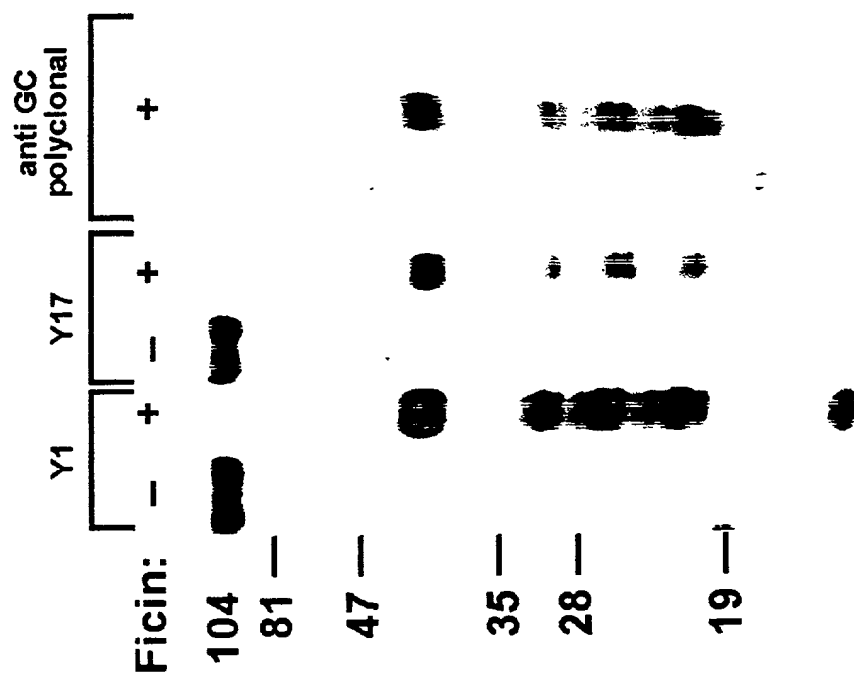
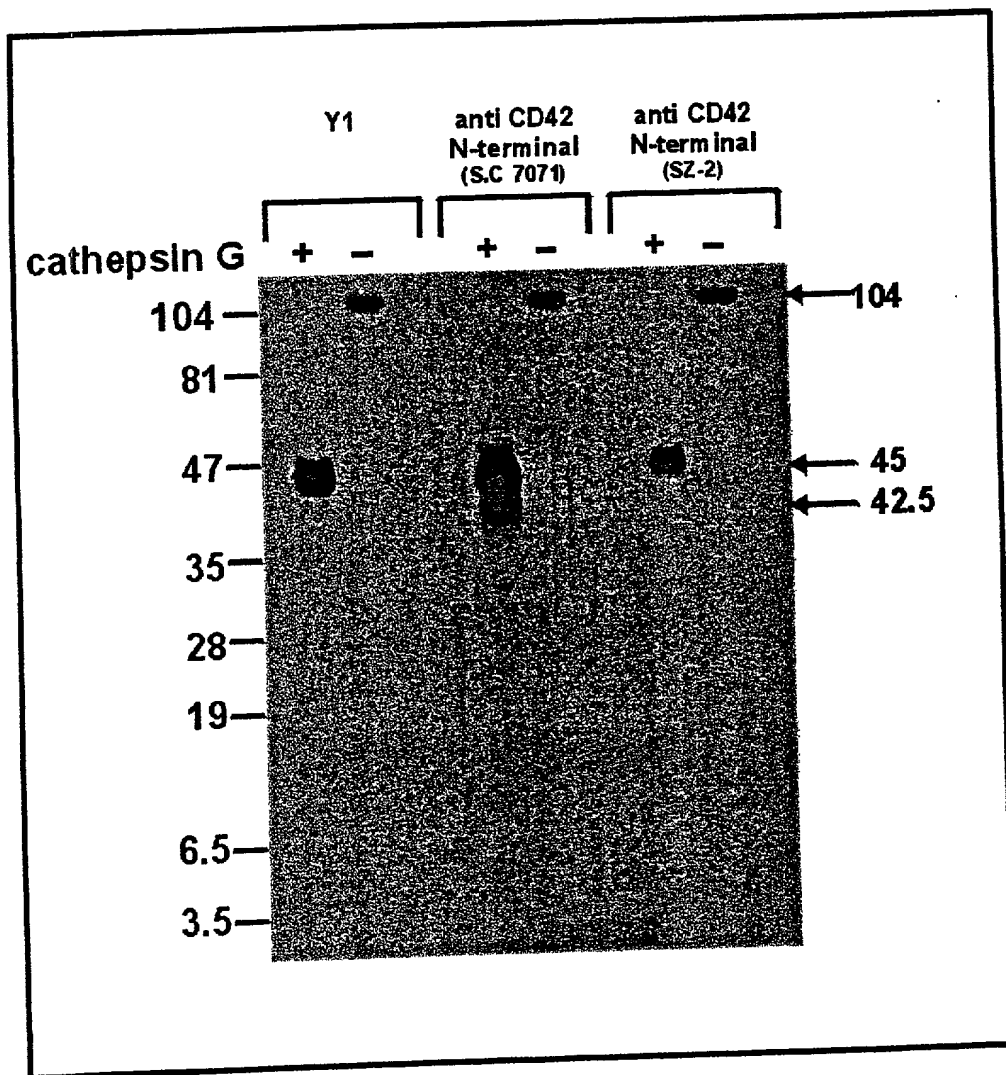
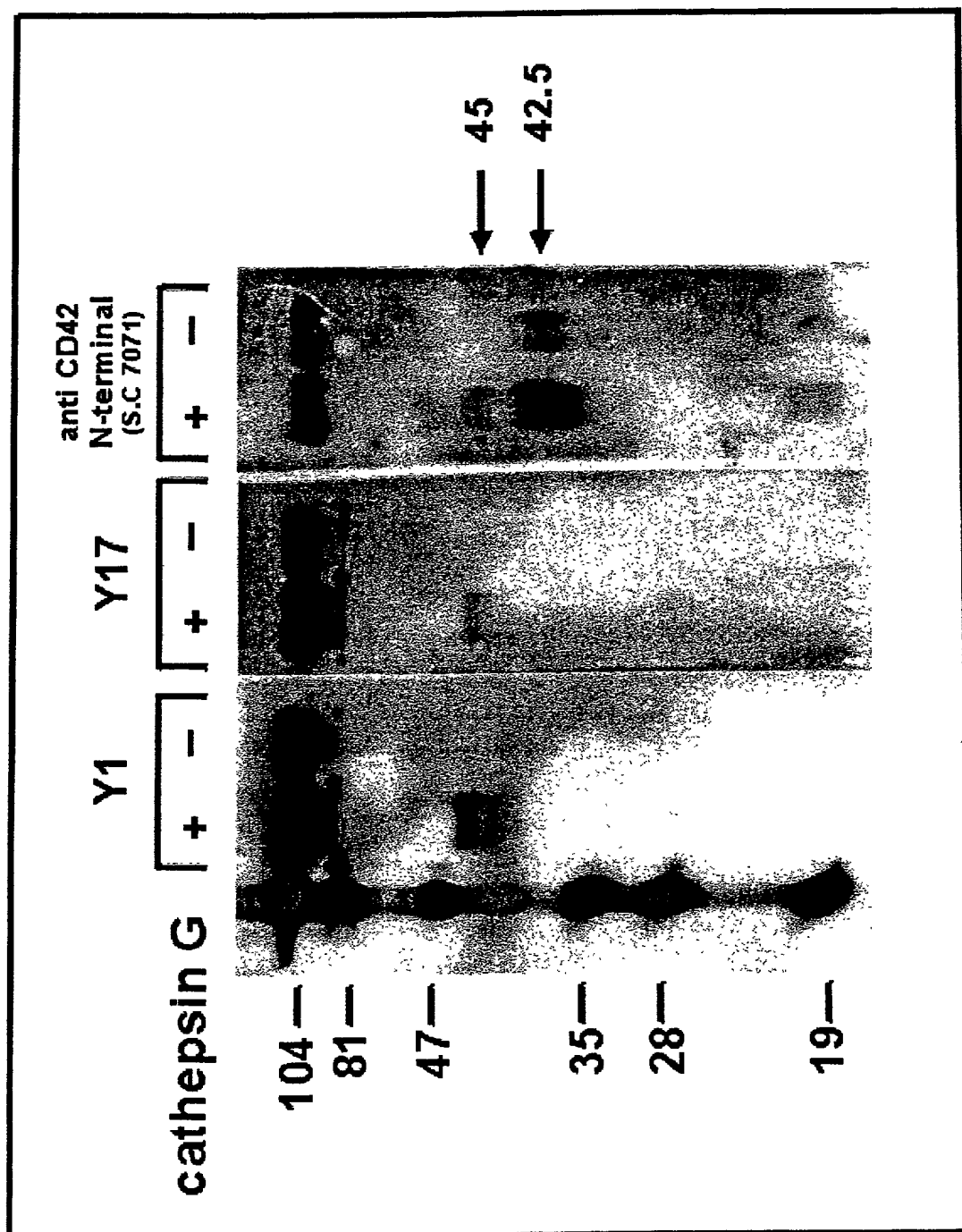


FIG. 10

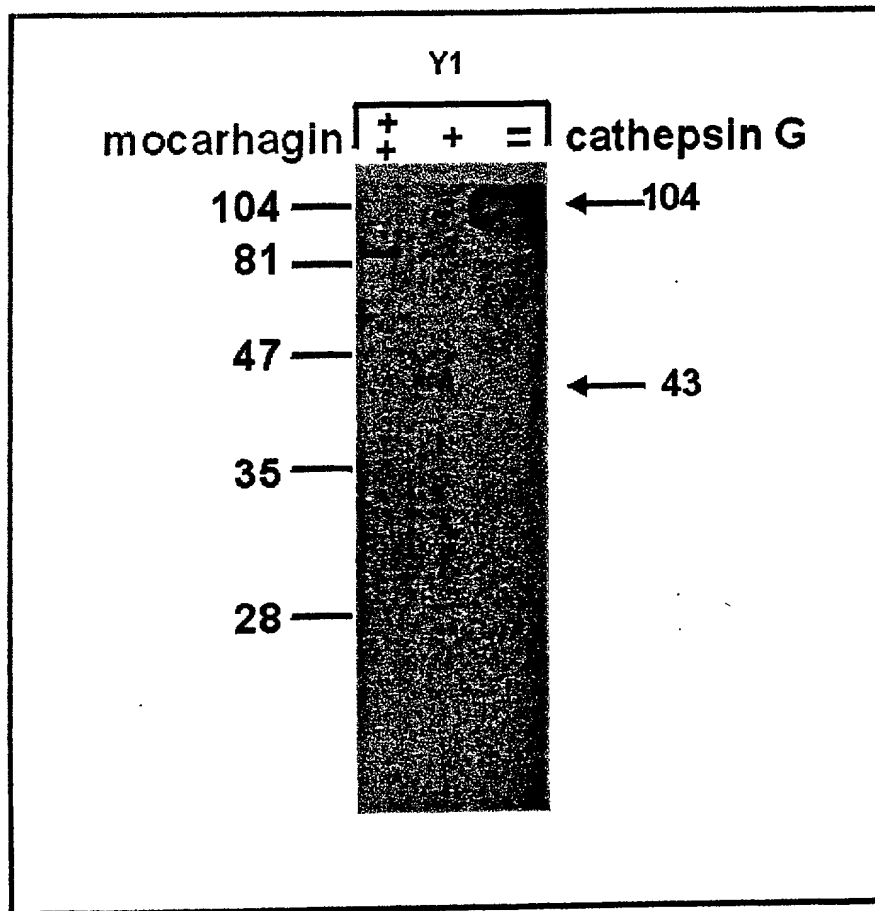
*FIG. 11*



# Y1 and Y17 reacts with larger cathepsin G cleaved platelets GPIb fragment



**FIG. 13**



# Cleavage of washed platelets by mocarhagin and cathepsin G

FIG. 14

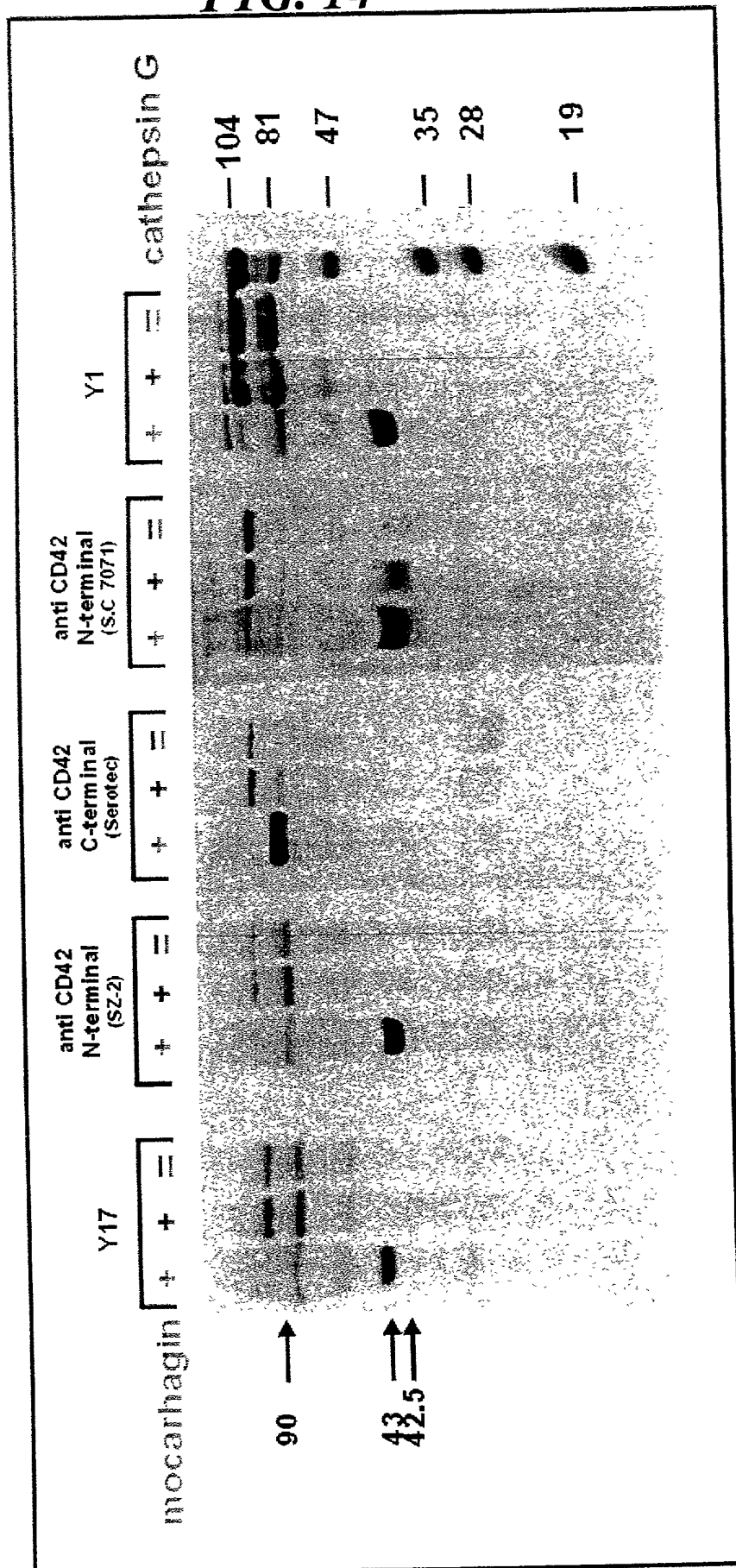
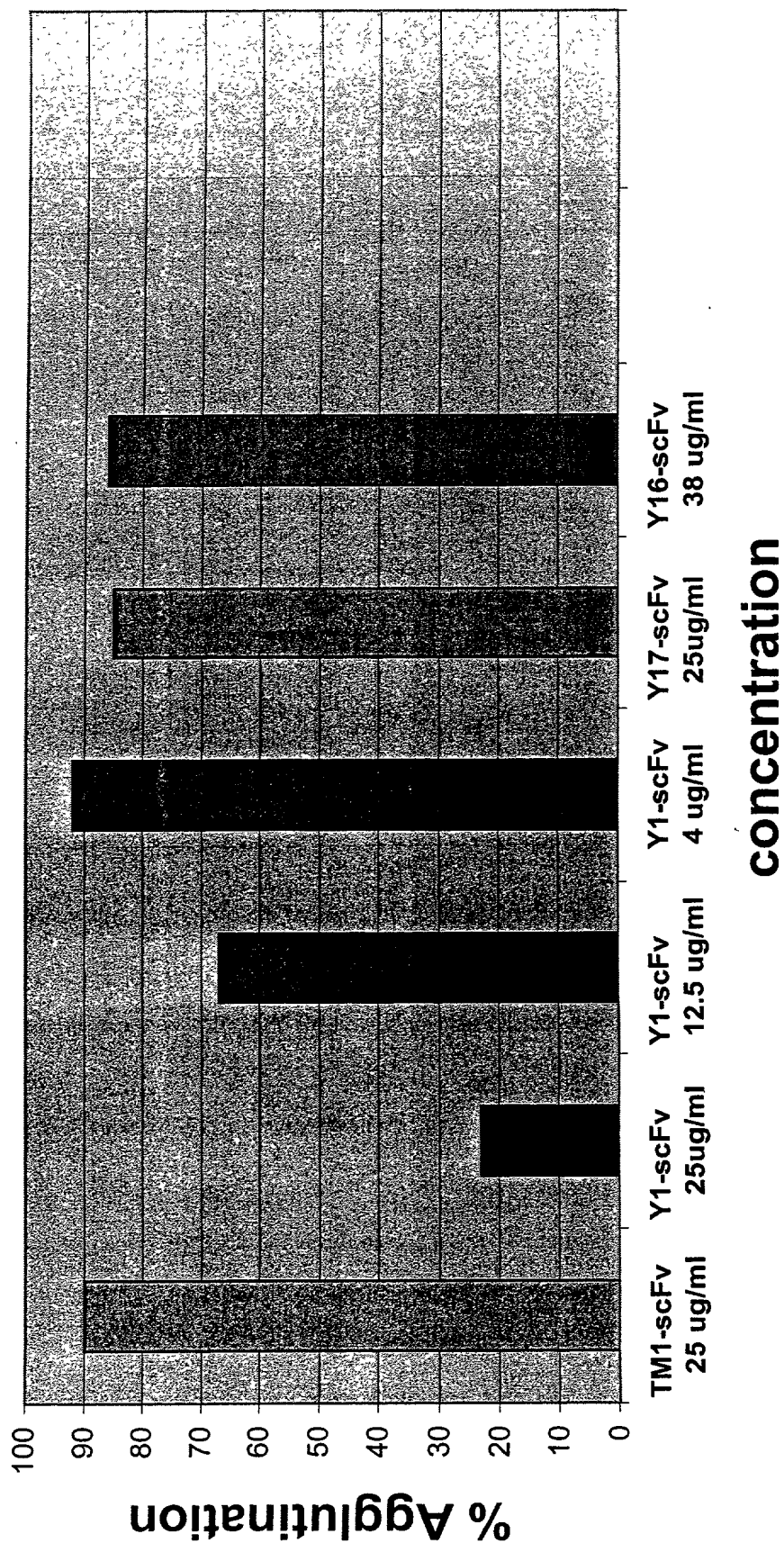
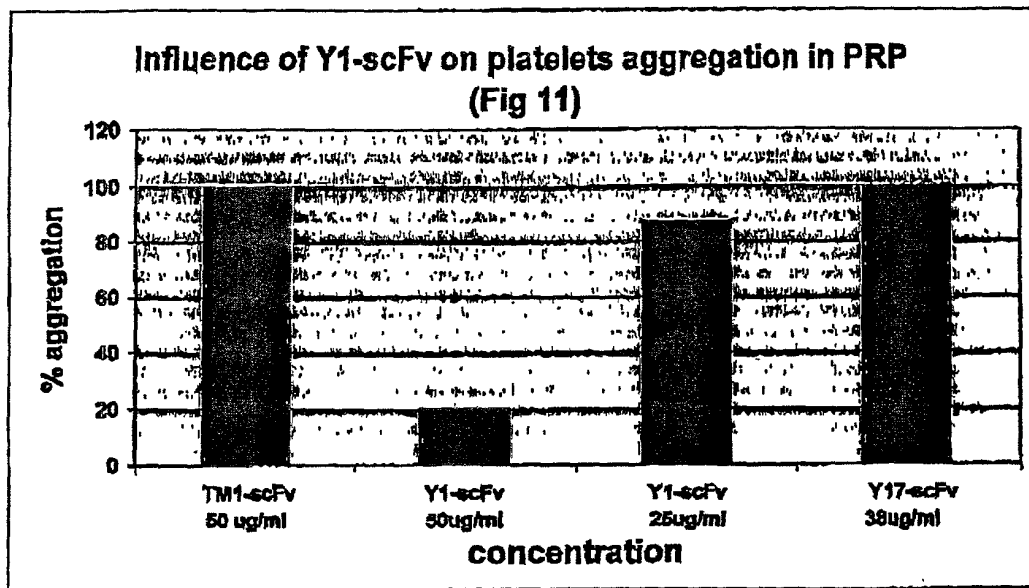


FIG. 15

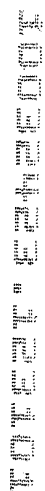
**Influence of Y1-scFv on platelets agglutination in washed platelets**



**FIG. 16**

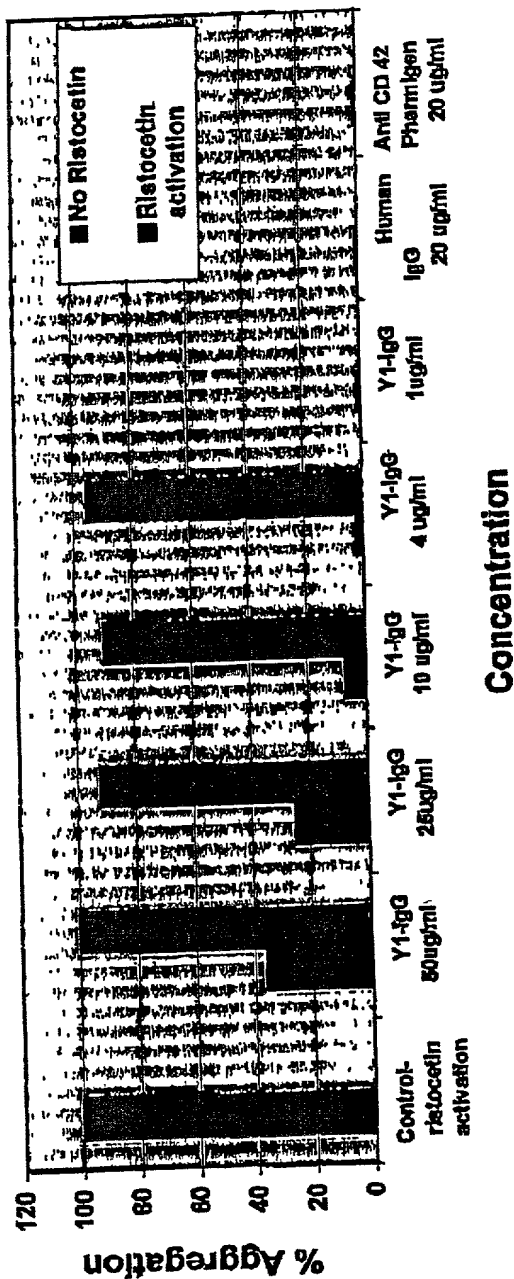




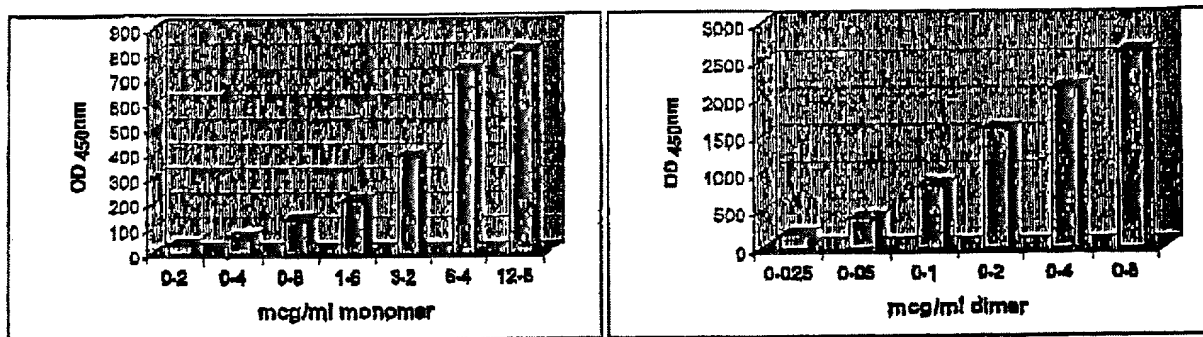
[illegible]

**FIG. 18**

**Induction of platelet aggregation by Y1-IgG in PRP**

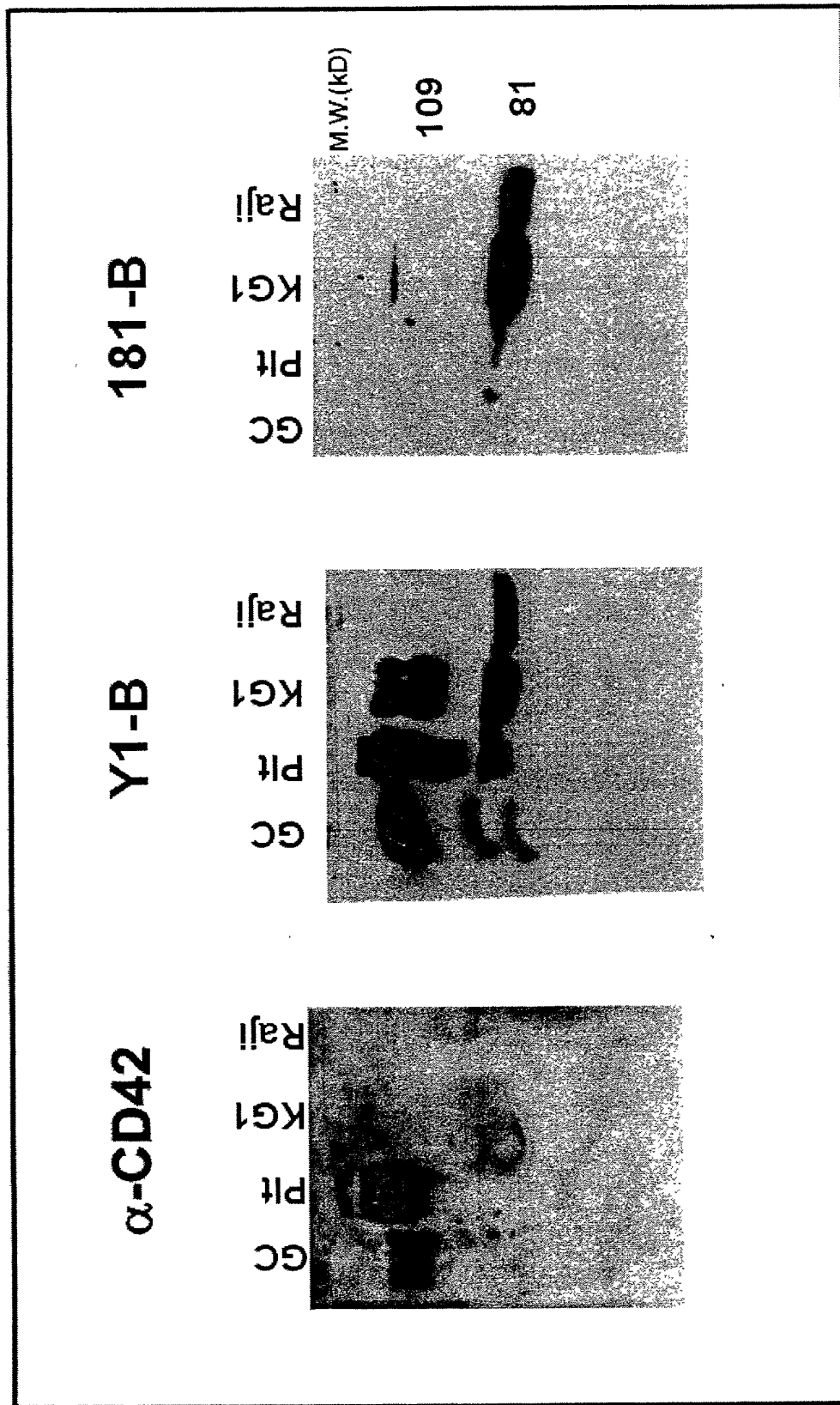


**FIG. 19**



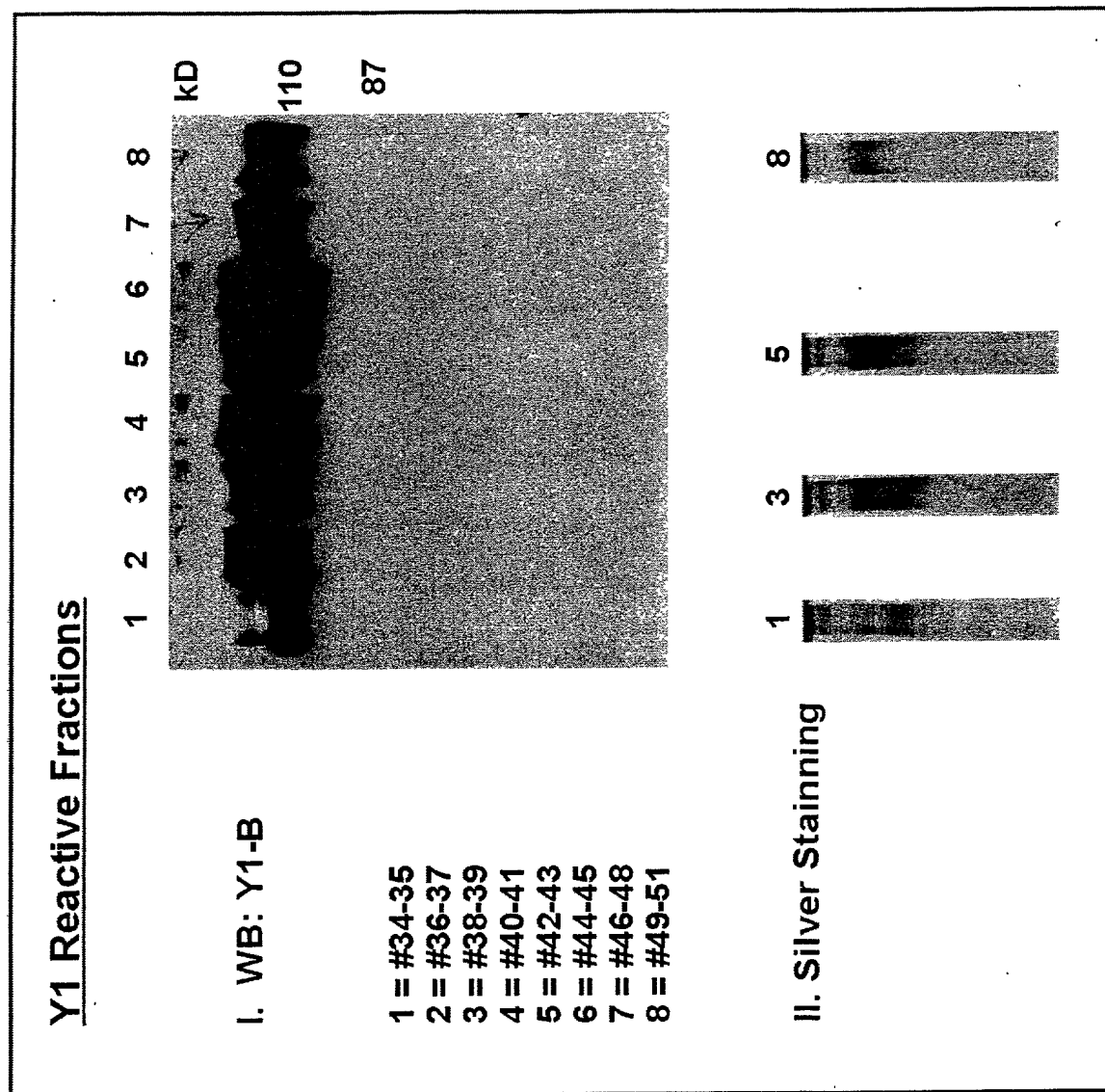
# Specificity of Binding of Y1 and $\alpha$ -CD42 (N1-19) to their Ligands

FIG. 20



# Y1-Ligand from KG1 membranes following Immuno-Precipitation with Y1: Purification on RP-HPLC

FIG. 21



# Effect of O-Sialo-Glycoprotein Endopeptidase on Y1 Binding

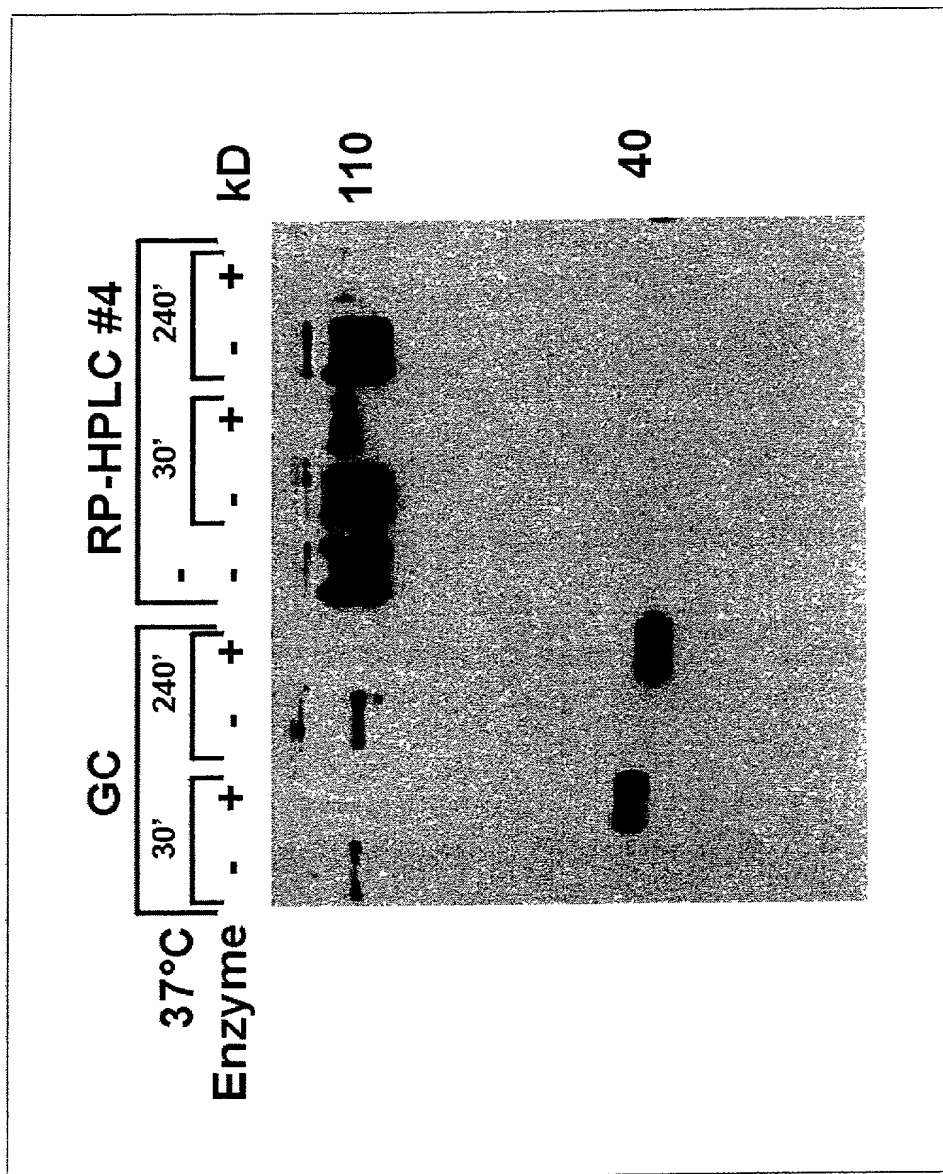


FIG. 22

# Effect of Aryl-Sulfatase on Binding of Y1: RP-HPLC(KG1) & H-B(Heparin-BSA)

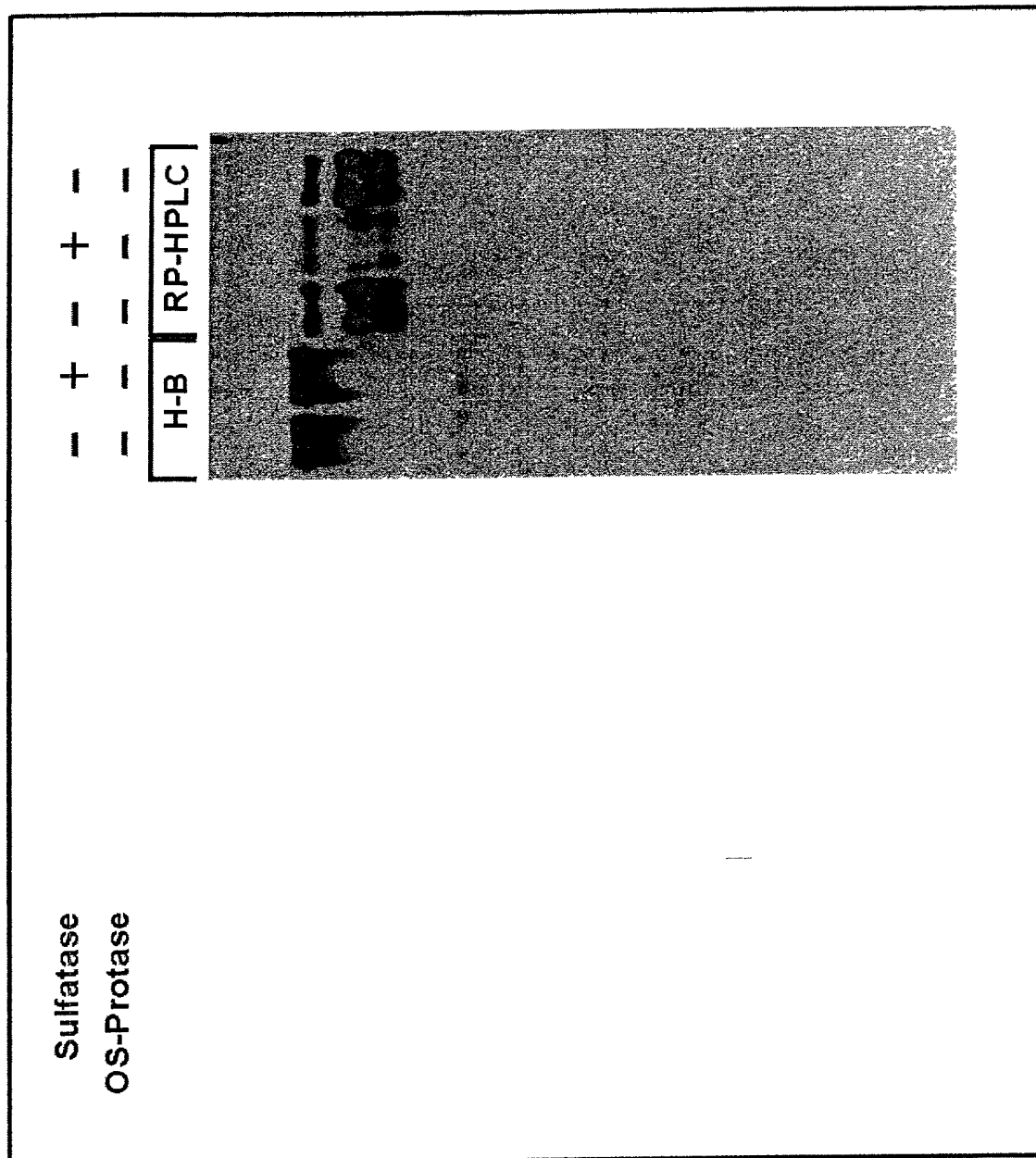


FIG. 23

# Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1

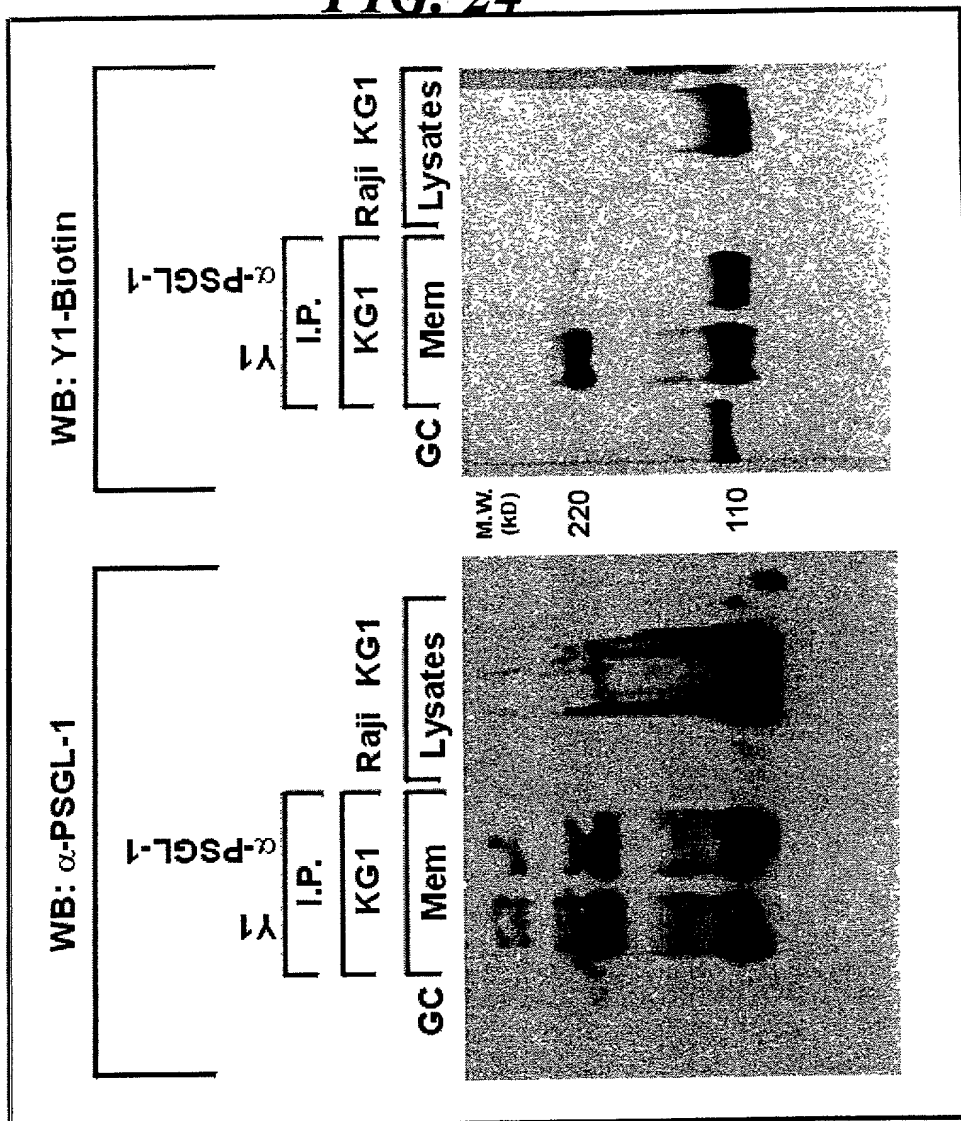
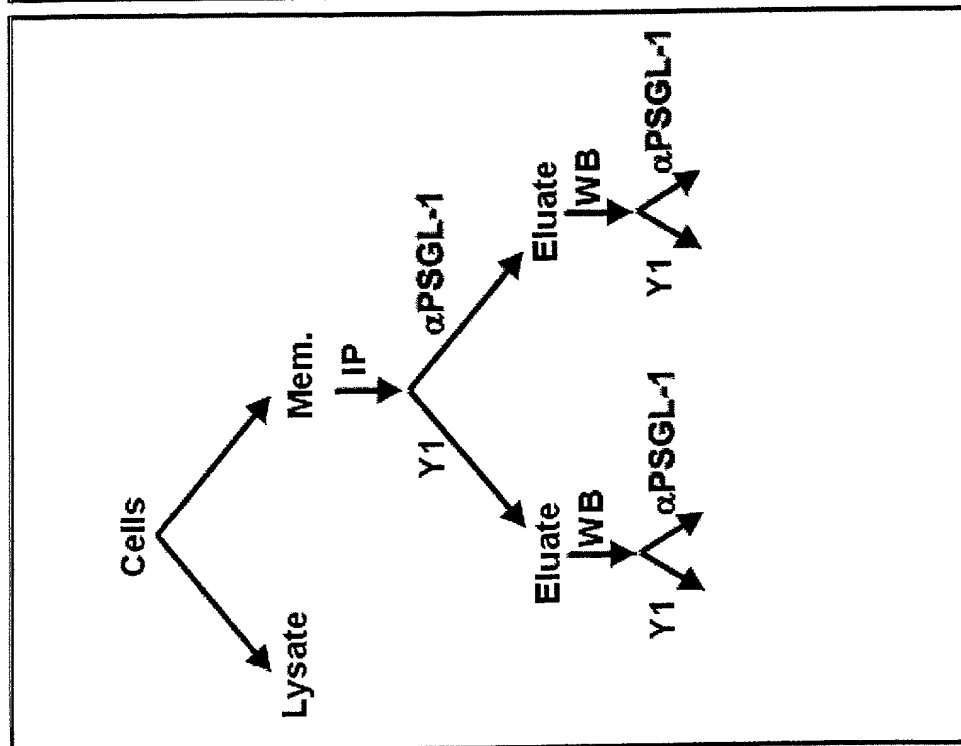


FIG. 24



**FIG. 25**

$\alpha$ -CD162 and Y1:  
Comparison between cells  
from AML patient and normal  
blood

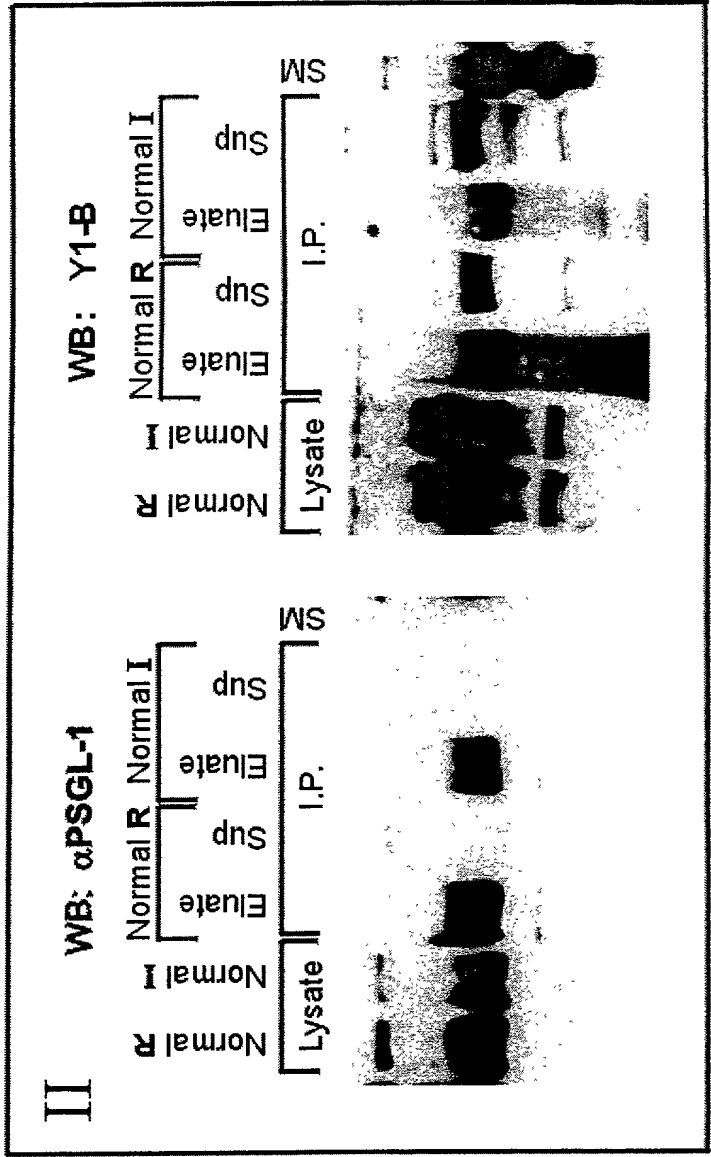
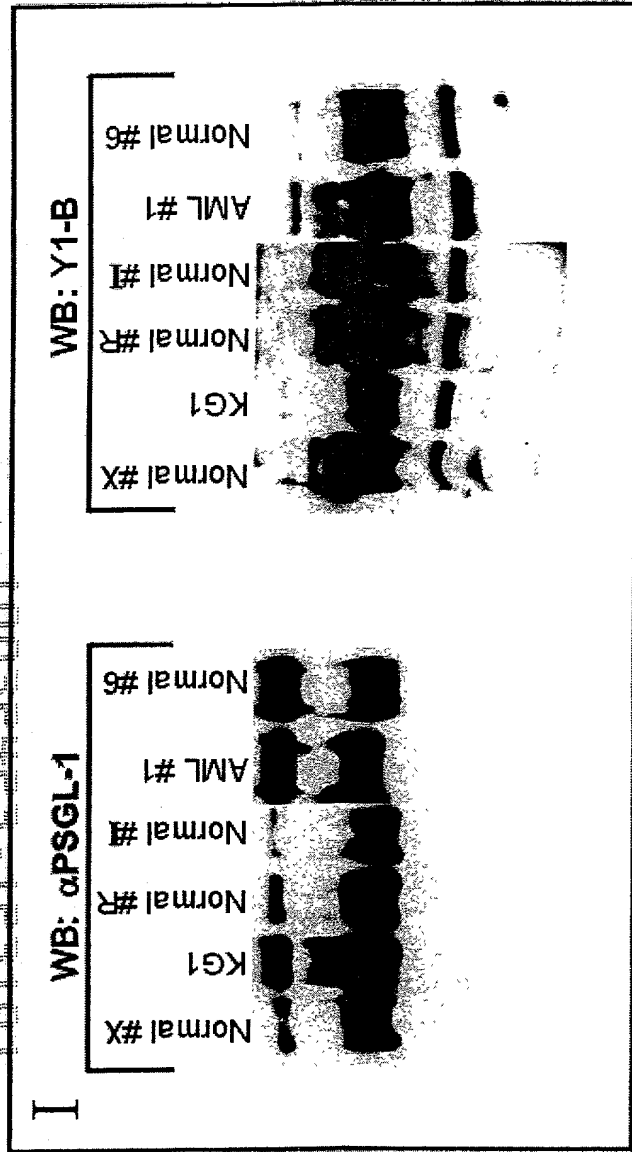
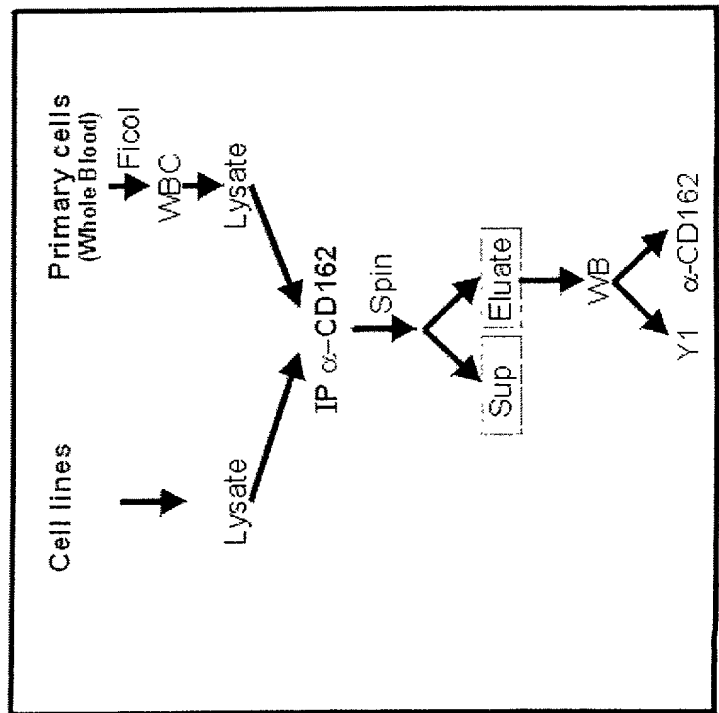
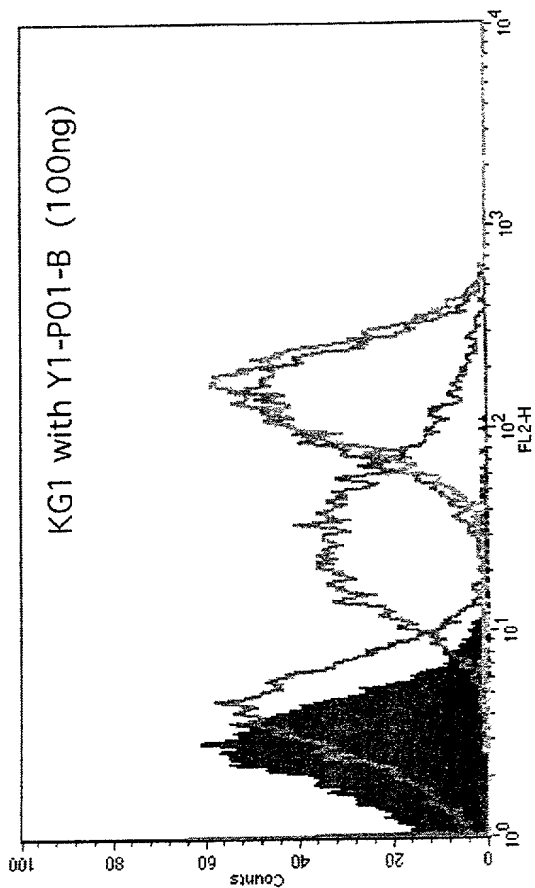
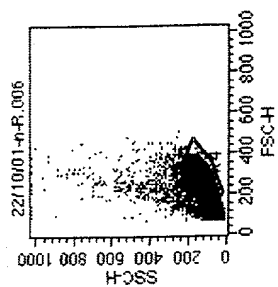
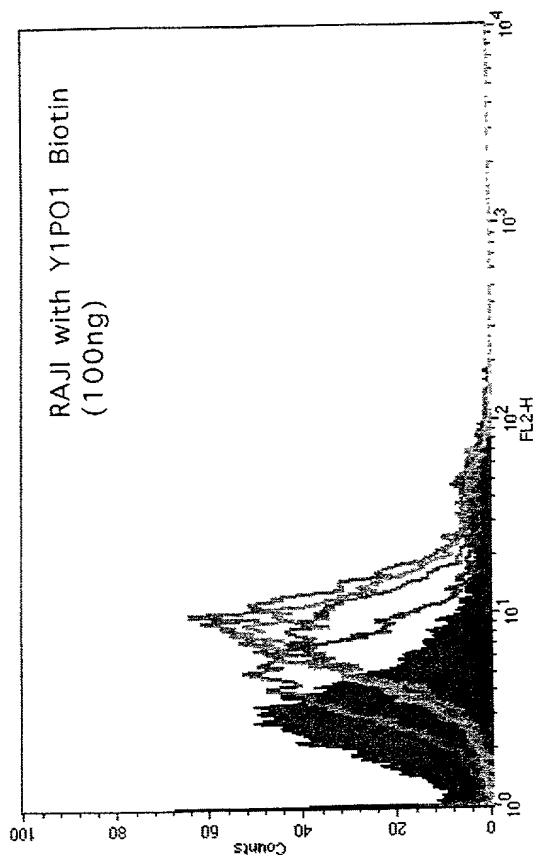
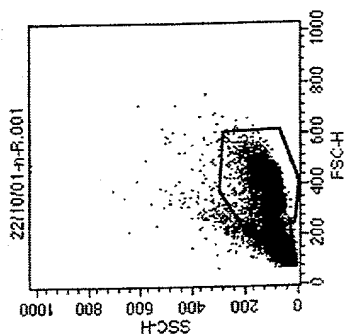


FIG. 26

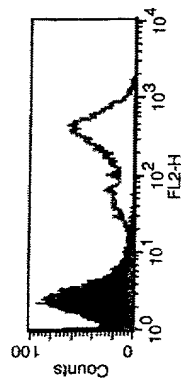


Key	Name	Parameter	Gate
	22/10/01-n-R.006	N01-B	
	22/10/01-n-R.007	P01-B	
	22/10/01-n-R.008	+KPL1	
	22/10/01-n-R.009	+PL1	
	22/10/01-n-R.010	+PL2	

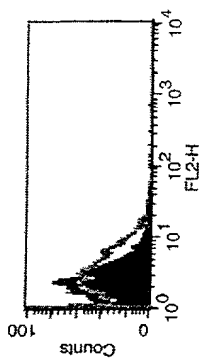
# Specificity of Y1 Binding: Analysis by FACS

- Binding of  
 $\alpha$  PSGL1  
( $\alpha$ CD162/KPL1);  
competition  
with Y1-IgG

KG-1



Raji



KG-1

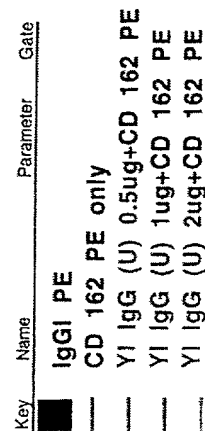
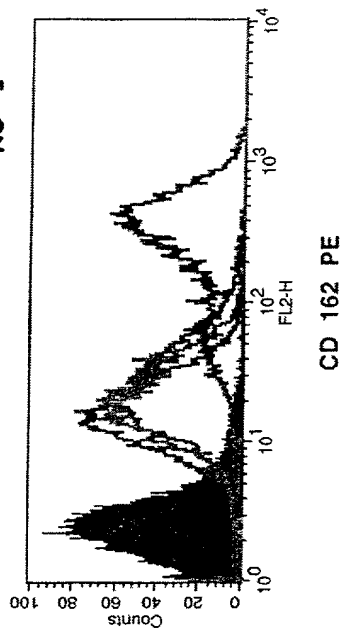


FIG. 27

# Specificity of Y1 Binding: Analysis by FACS

- Binding of  
Y1-IgG;  
competition  
with  $\alpha$ PSGL-1  
(CD162 /KPL1)

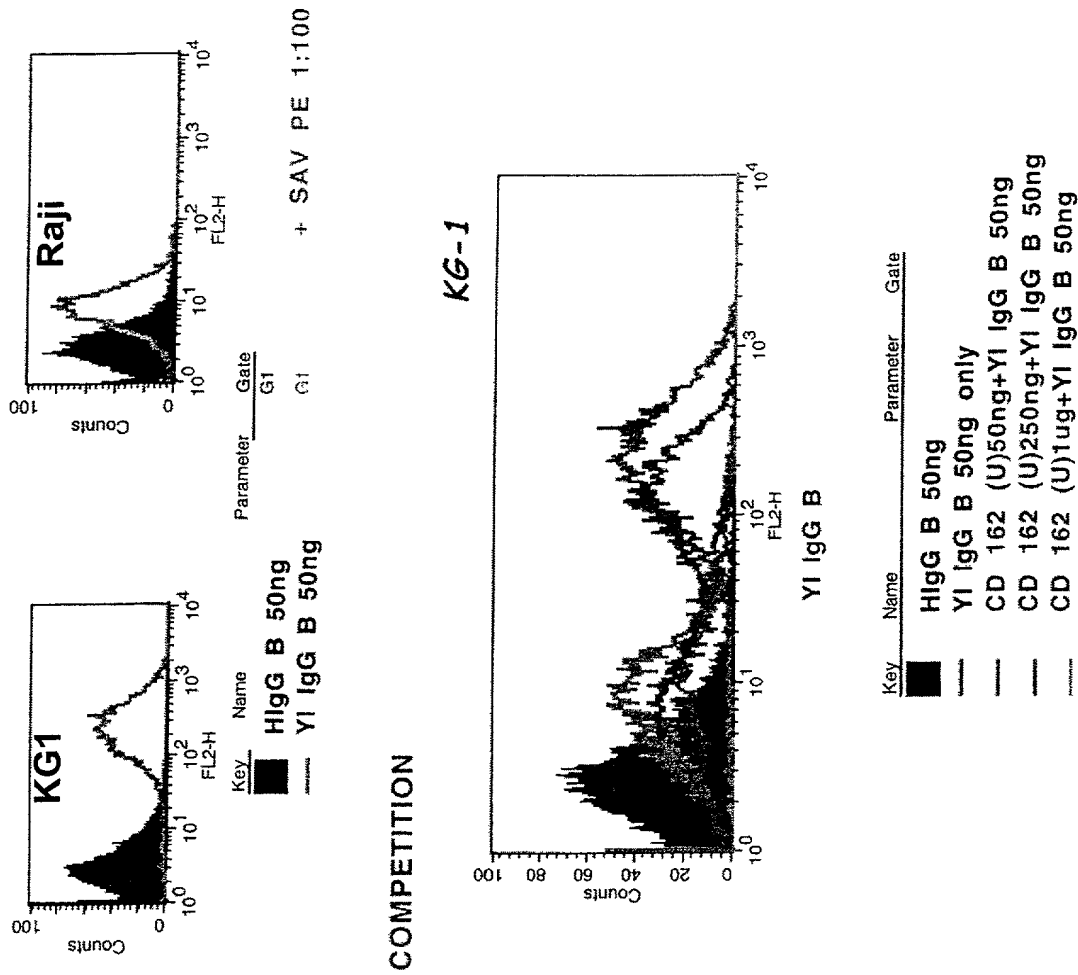
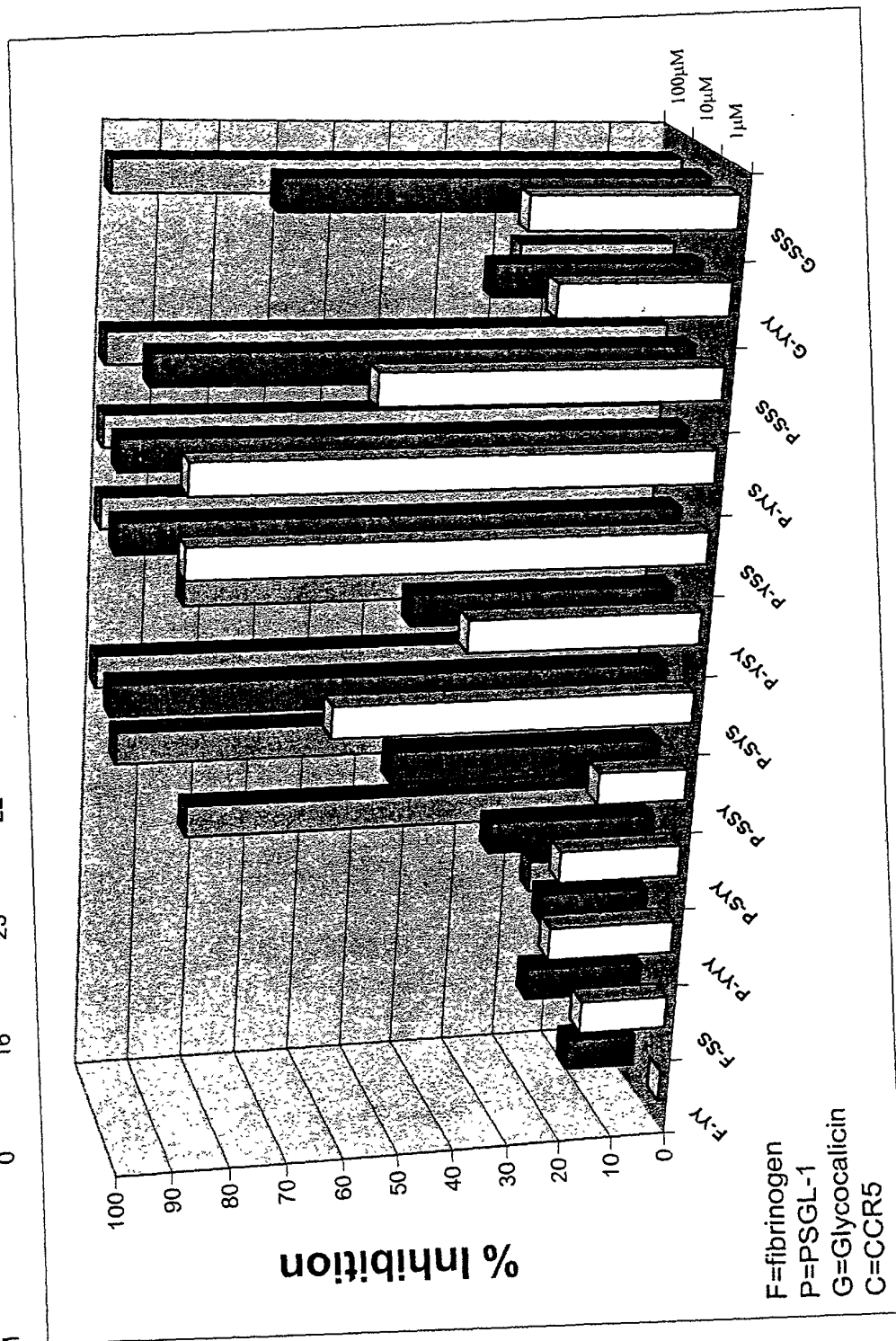


FIG. 28

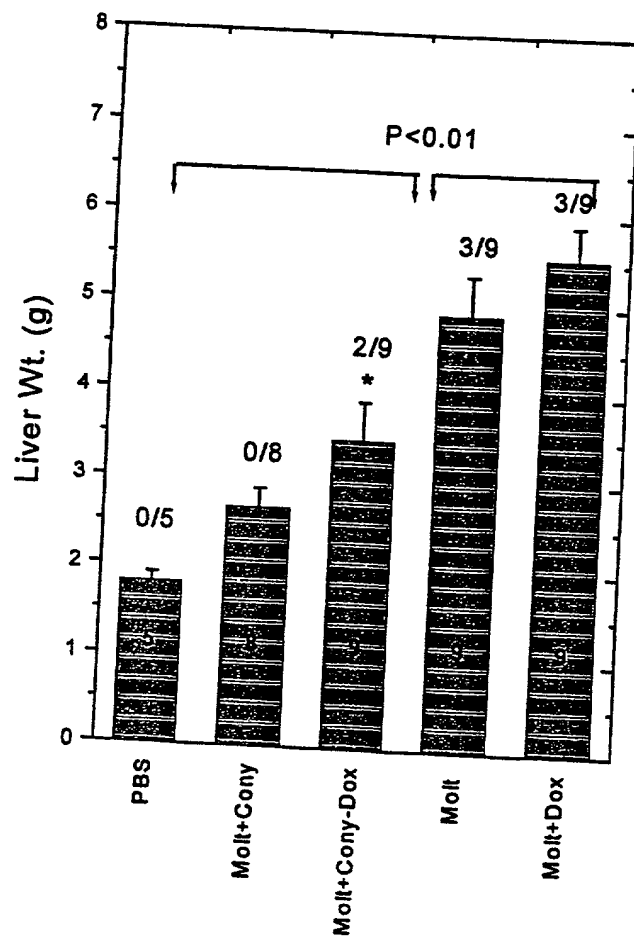
FIG. 29

FOOTNOTES

	F-YY	F-SS	P-YYY	P-SYY	P-SSY	P-SYS	P-YSY	P-YSS	P-YYs	P-SSS	G-YYY	G-SSS
100μM	0	0	18	83	96	100	100	85	100	100	100	100
10μM	13	22	20	31	50	100	100	48	100	95	37	75
1μM	0	16	23	22	16	65	65	42	91	60	30	36

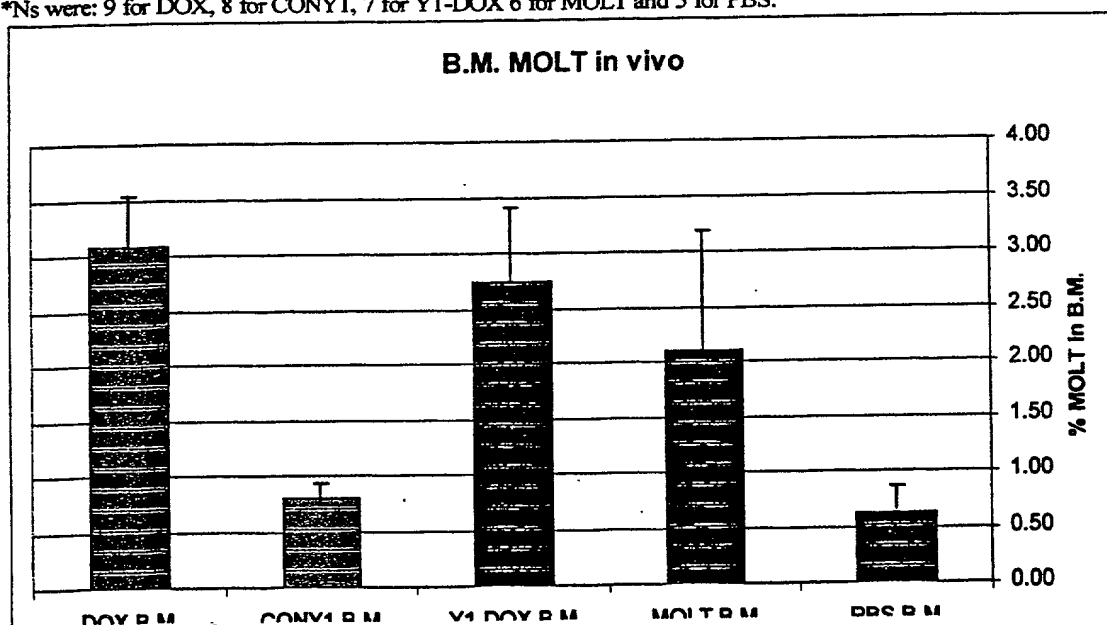


**FIG. 30**

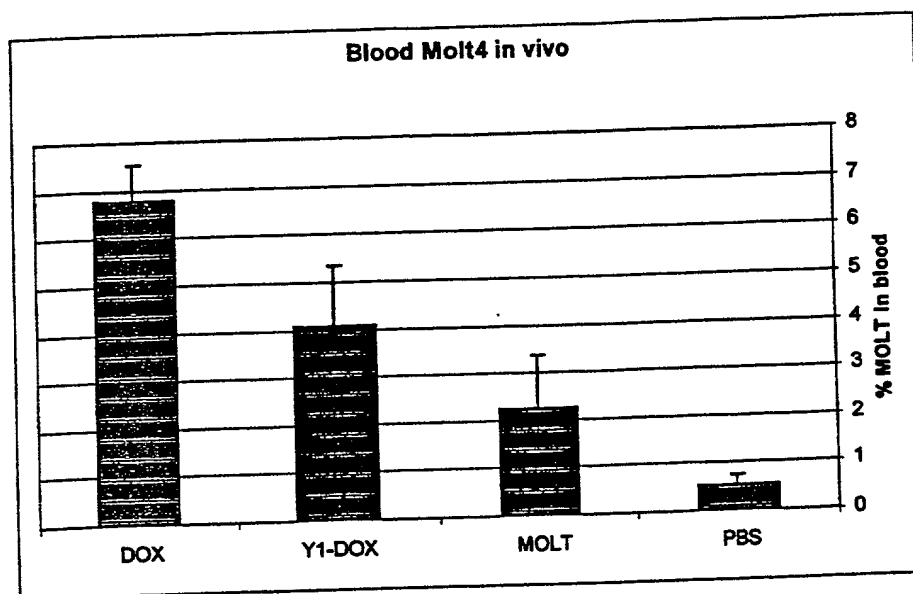


**FIG. 31**

\*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.



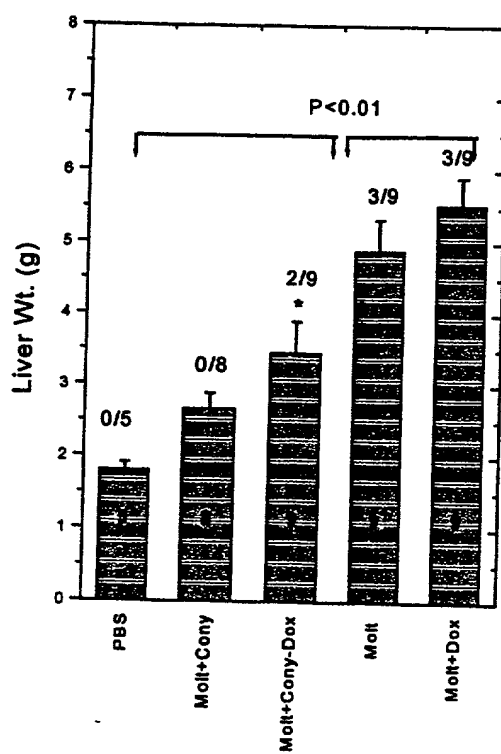
**FIG. 32**



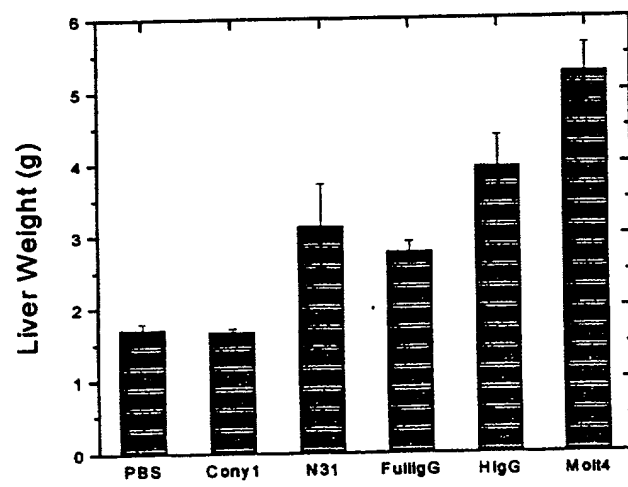
**\*\*Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.**



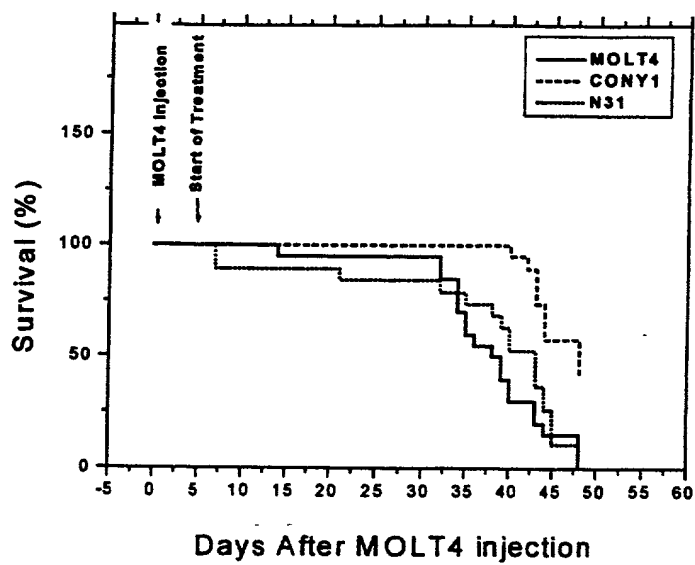
**FIG. 33**



**FIG. 34**

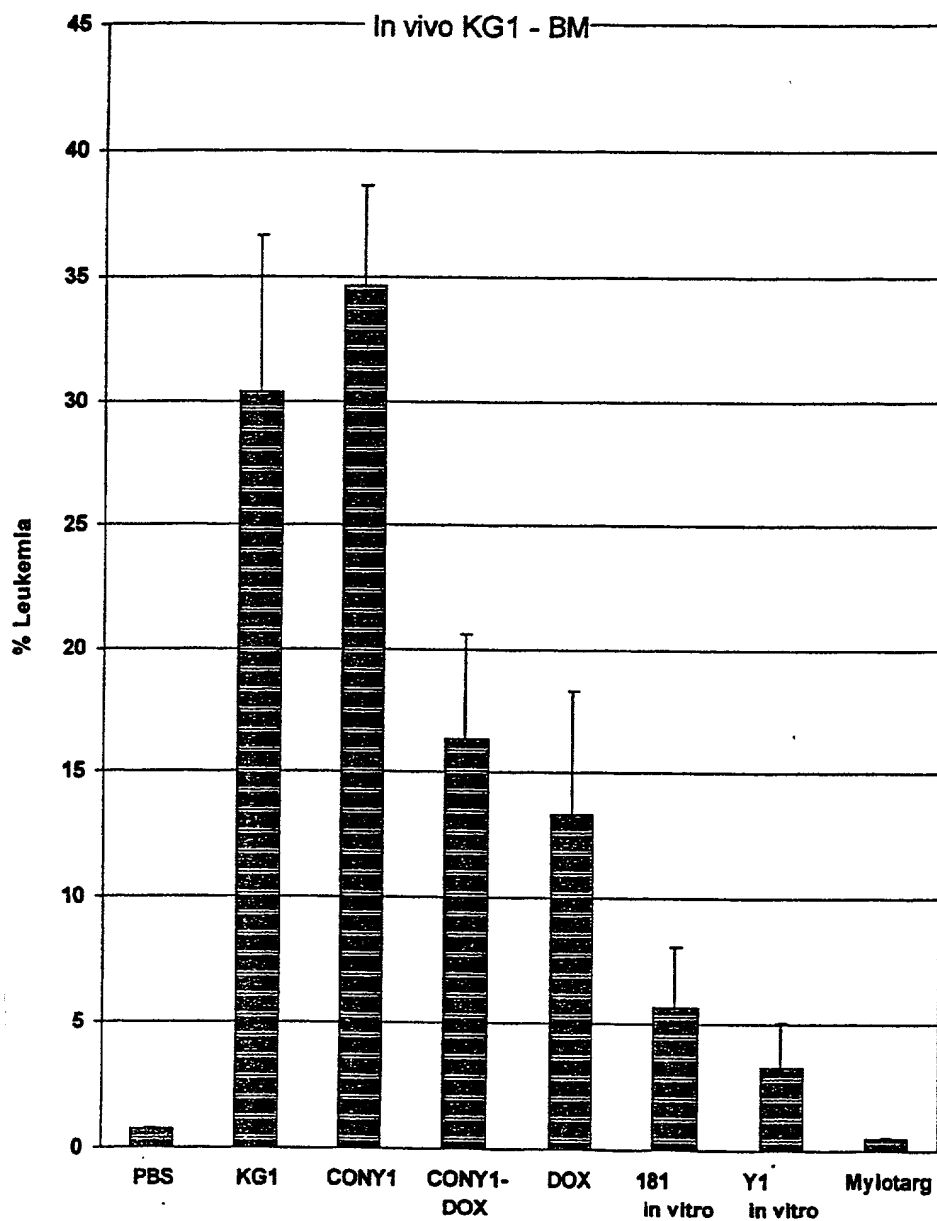


**FIG. 35**



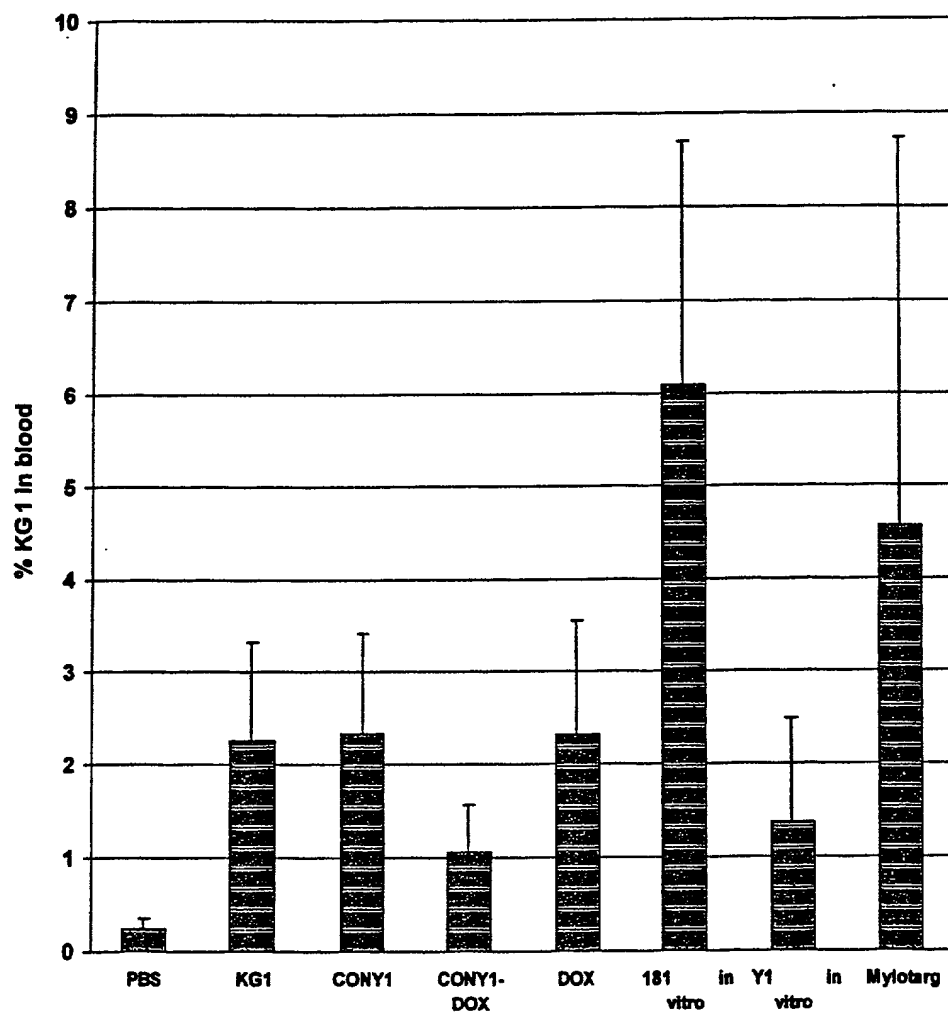
**FIG. 36**

\*\*\*Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.



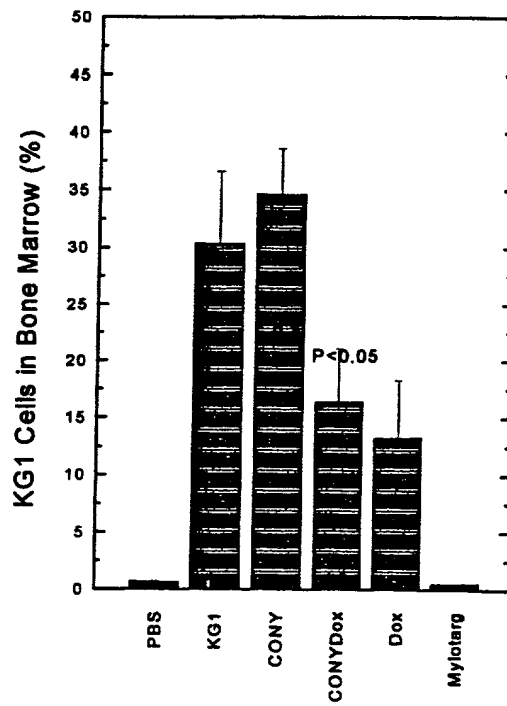
**FIG. 37**

**In vivo KG1 - Blood**

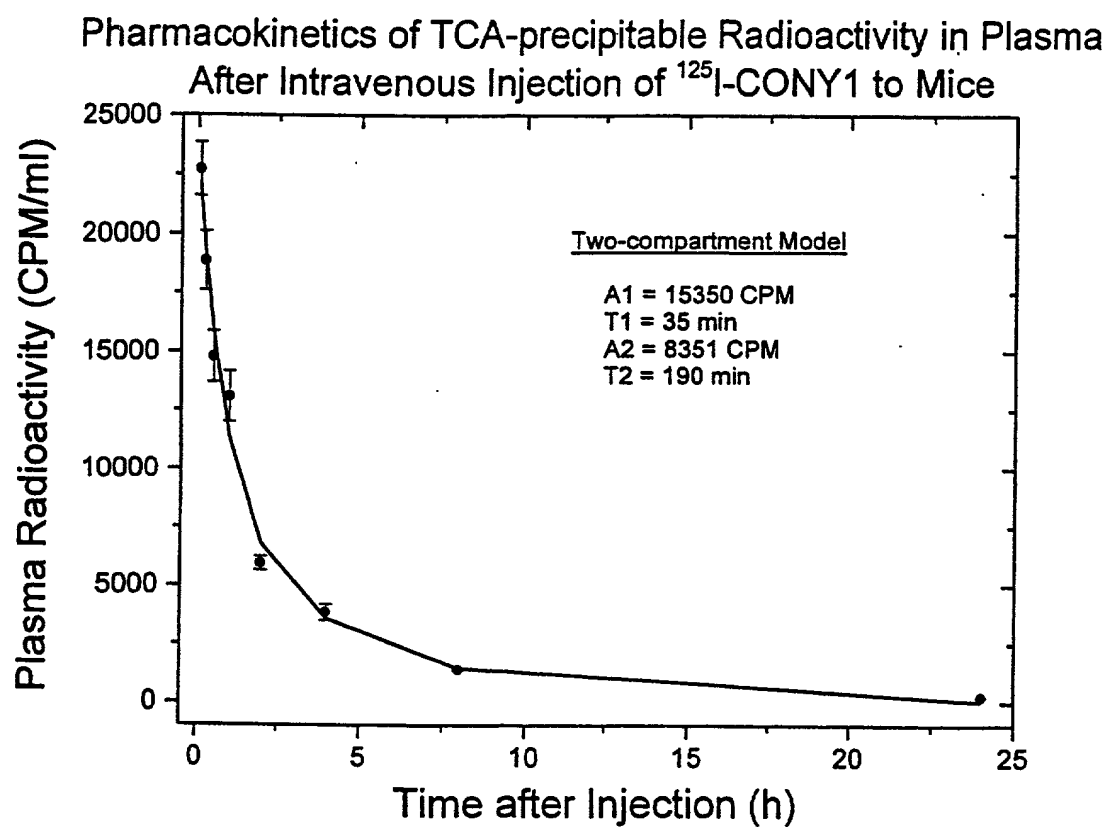


\*\*\*\*Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.

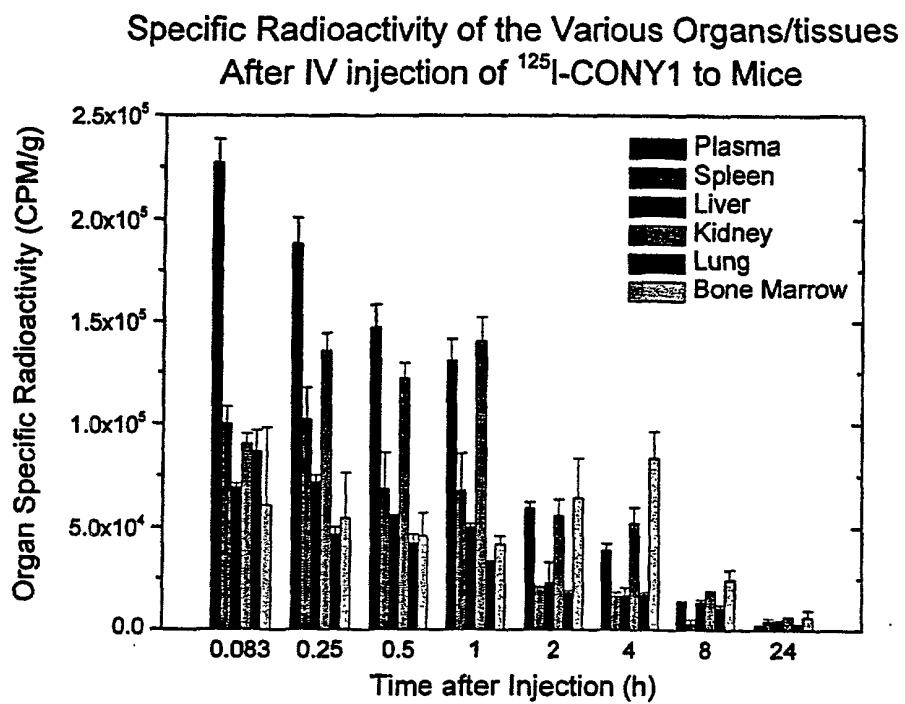
**FIG. 38**



**FIG. 39**



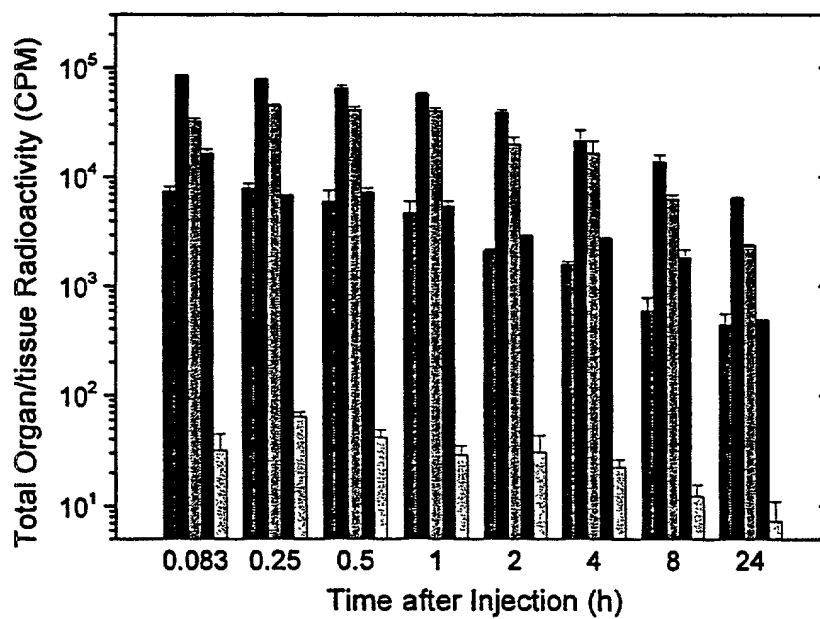
Variable	Mean	Standard Deviation	Minimum	Maximum
Age	34.5	10.2	22	55
Gender	0.5	0.5	0	1
Marital Status	0.7	0.5	0	1
Education	12.5	1.5	10	15
Income	3500	1500	1000	7000
Health	0.8	0.3	0	1
Stress	4.5	1.5	1	7
Depression	2.5	1.0	1	4
Life Satisfaction	5.5	1.0	1	7
Resilience	3.5	1.0	1	5
Optimism	4.0	1.0	1	5
Gratitude	4.5	1.0	1	5
Forgiveness	4.0	1.0	1	5
Self-Compassion	4.0	1.0	1	5
Emotional Regulation	4.0	1.0	1	5
Prosocial Behavior	4.0	1.0	1	5
Life Purpose	4.0	1.0	1	5
Meaning in Life	4.0	1.0	1	5
Existential Well-being	4.0	1.0	1	5
Transcendental Well-being	4.0	1.0	1	5
Overall Well-being	4.0	1.0	1	5



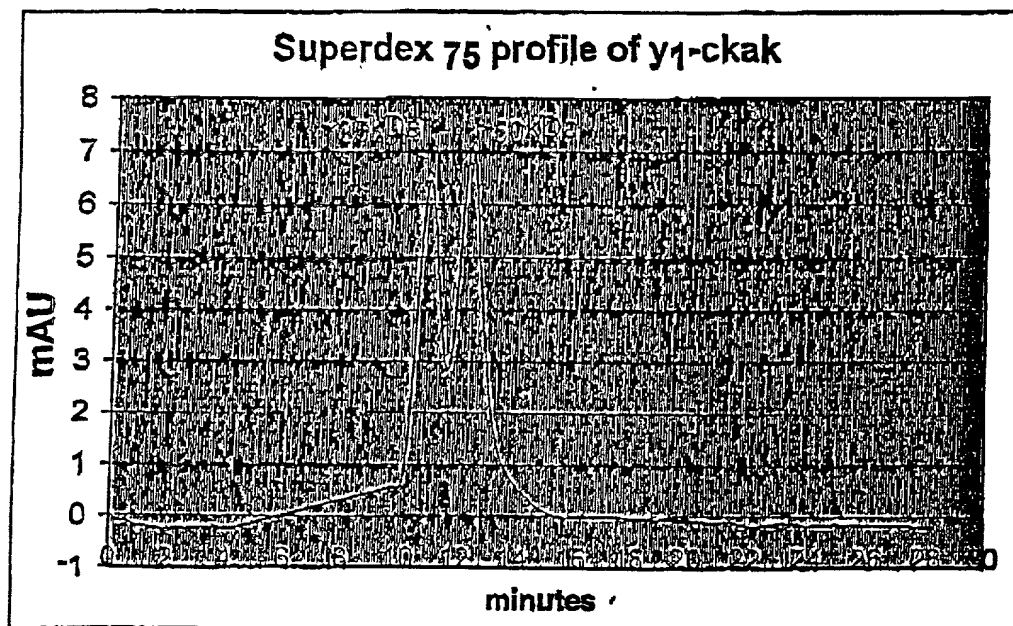


**FIG. 41**

Distribution of Radioactivity in Body organs after  
Injection of  $^{125}\text{I}$ -CONY1 to Mice



**FIG. 42**



10032423-123101  
TOTCCT-22422001

**FIG. 43**

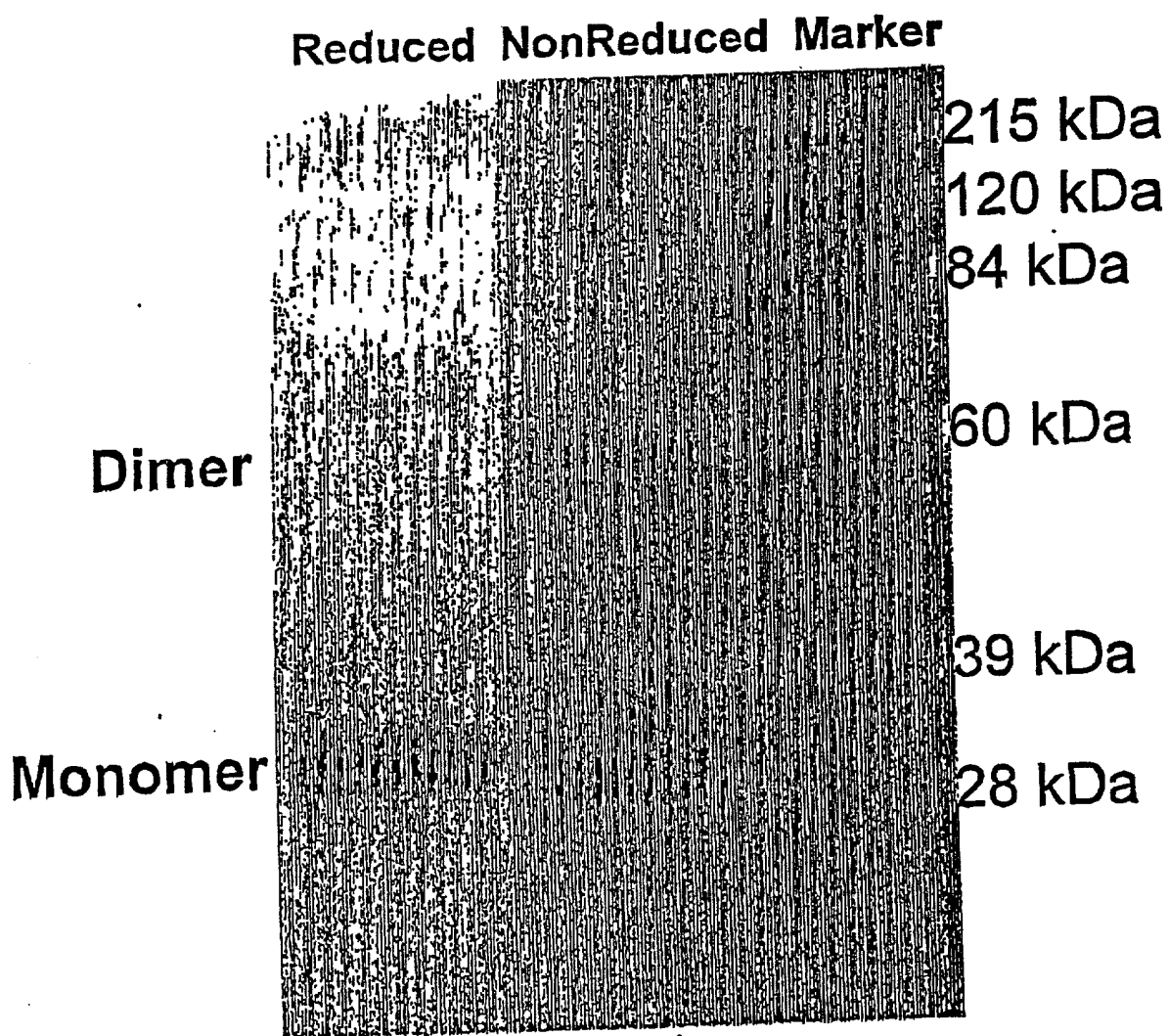
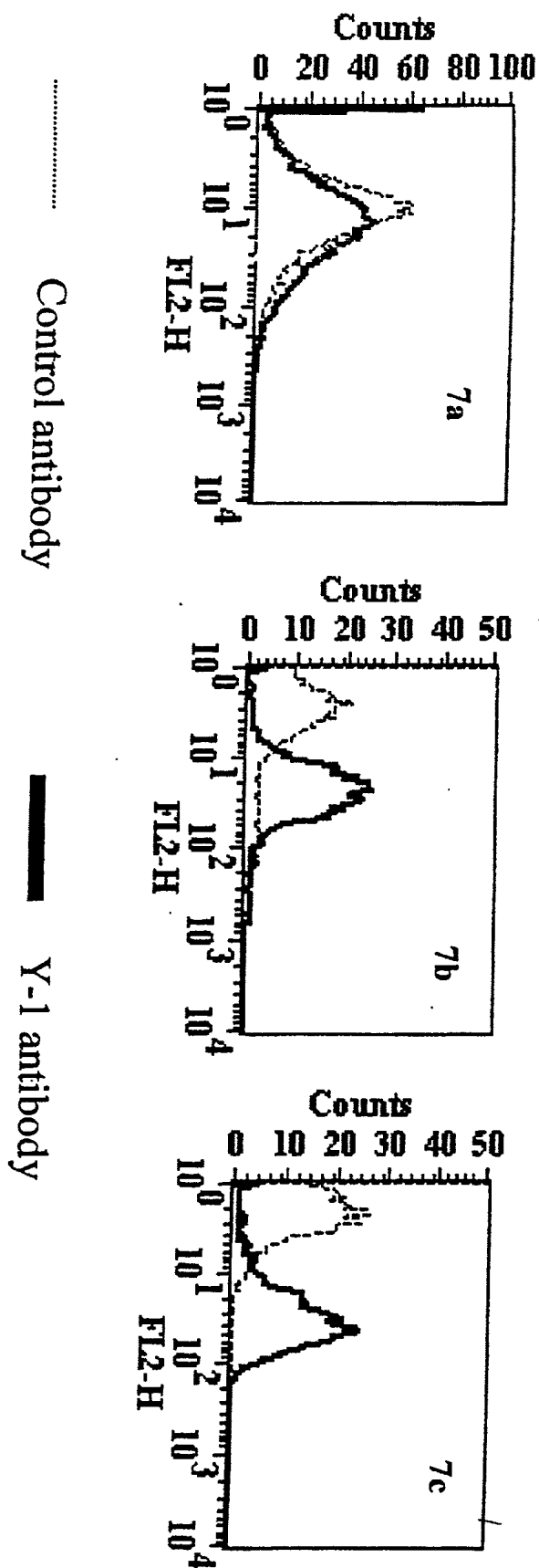


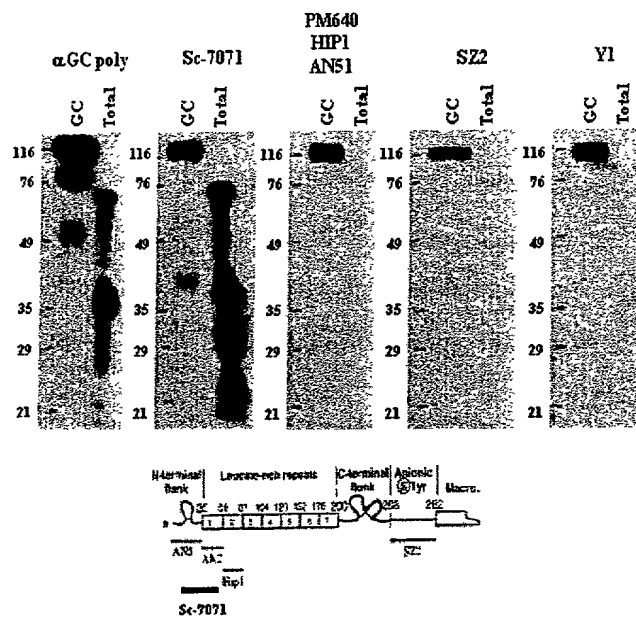
FIG. 44



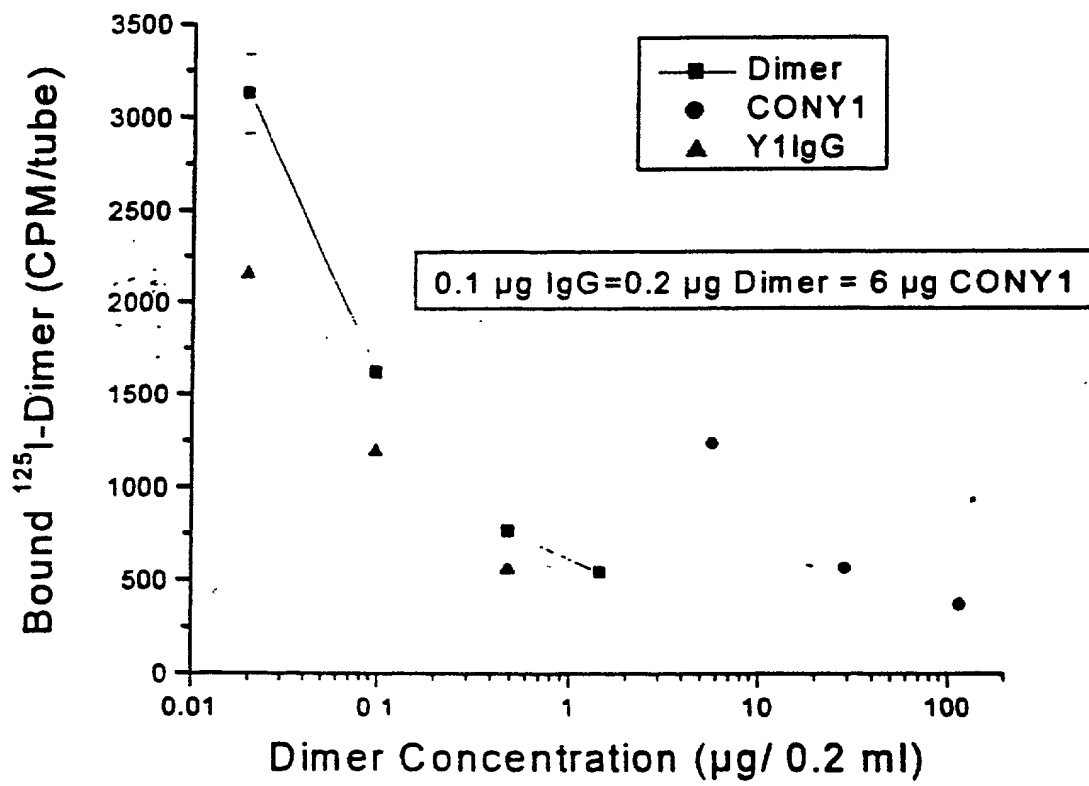
10032423-133401

# FIG. 45

## Epitopes of anti-GPIb $\alpha$ antibodies



**FIG. 46**



**FIG. 47**

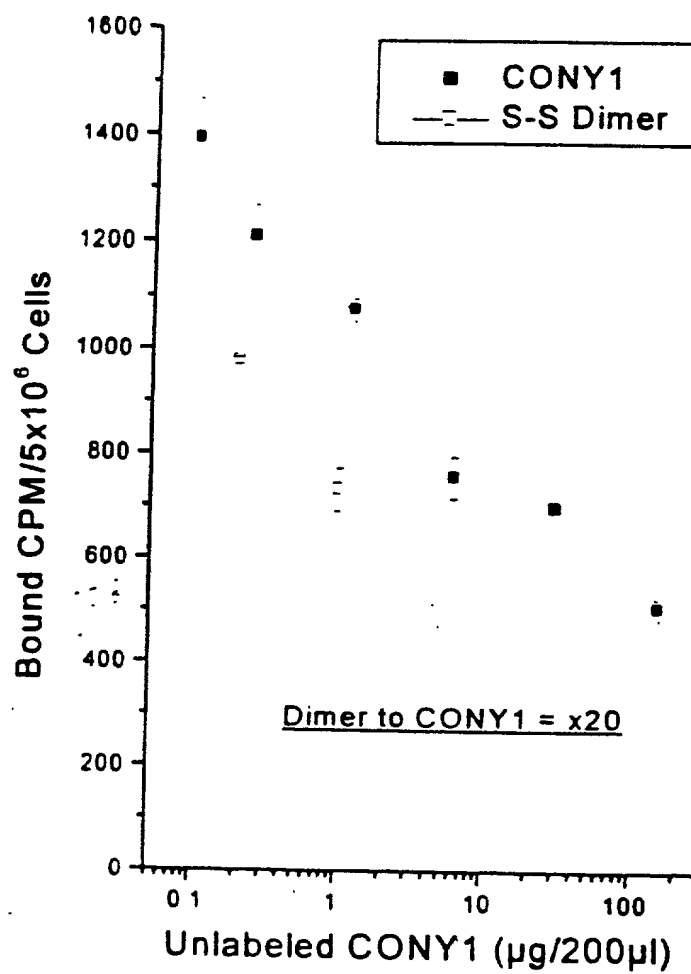


FIG. 48A: The ORF and Amino Acid Sequence of Y1-HC

SEQ ID NO: 205 (nucleic acid sequence): SEQ ID NO: 206 (amino acid sequence)

1	ATGGCCTGGGCTCTGCTGCTCCTOACCCTCCTCACTCAGGACACAGGGTCTGGGCCGAT
1	<u>M A W A L L L L T L L T Q D T G S W A D</u>
61	ATCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCTCC
21	I Q L V E S G G G V V R P G G S L R L S
121	TGTGCAGCCTCTGGATTCACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41	C A A S G F T F D D Y G M S W V R Q A P
181	GGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61	G K G L E W V S G I N W N G G S T G Y A
241	GACTCTGTGAAGGGCCGATTACCATCTCTAGAGACAACGCCAAGAACTCCCTGTATCTG
81	D S V K G R F T I S R D N A K N S L Y L
301	CAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101	Q M N S L R A E D T A V Y Y C A R M R A
361	CCTGTGATTGTTGGGGCCAAAGGTACCCTGGTCACCGTCTCGAGTGCTTCCACCAAGGGCCCA
121	P V I W G Q G T L V T V S S A S T K G P
421	TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141	S V F P L A P S S K S T S G G T A A L G
481	TGCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGCGCCCTG
161	C L V K D Y F P E P V T V S W N S G A L
541	ACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGC
181	T S G V H T F P A V L Q S S G L Y S L S
601	AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201	S V V T V P S S S L G T Q T Y I C N V N
661	CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAT
221	H K P S N T K V D K R V E P K S C D K T
721	CACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGACTGTGAGTCTTCOTCTTC
241	H T C P P C P A P E L L G G P S V F L F
781	CCCCCAAACCCAAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTG
261	P P K P K D T L M I S R T P E V T C V V
841	GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGAG
281	V D V S H E D P E V K F N W Y V D G V E
901	GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301	V H N A K T K P R E E Q Y N S T Y R V V
961	AGCGTCCTCACCGTCTGACACAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
321	S V L T V L H Q D W L N G K E Y K C K V
1021	TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCC
341	S N K A L P A P I E K T I S K A K G Q P
1081	OGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGAGGAGATGACCAAGAACAGGTC
361	R E P Q V Y T L P P S R E E M T K N Q V
1141	AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381	S L T C L V K G F Y P S D I A V E W E S
1201	AATGGGCAGCCGGAGAACAACACTACAAGACCACGTCTCCCGTGTGACTCCGACGGCTCC
401	N G Q P E N N Y K T T S P V L D S D G S
1261	TTCTTCCTCTATAGCAAGCTCACCGTGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421	F F L Y S K L T V D K S R W Q Q G N V F
1321	TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441	S C S V M H E A L H N H Y T Q K S L S L
1381	TCTCTGGGTAAATGA
461	S L G K *

10032423-133101



FIG. 48B: The ORF and Amino Acid Sequence of Y1-LC

SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)

1	ATGGCCTGGGCTCTGCTGCTCCTCACCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1	<u>M A W A L L L L T L L T Q D T G S W A D</u>
61	GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21	A E L T Q D P A V S V A L G Q T V R I T
1212	TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41	C Q G D S L R S Y Y A S W Y Q Q K P G Q
181	GGCCCTGTACTTGTCTATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTG
161	A P V L V I Y G K N N R P S G I P D R F
241	TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81	S G S S S G N T A S L T I T G A Q A E D
301	GAGGCTGACTATTACTGTAAGTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGA
101	E A D Y Y C N S R D S S G N H V V F G G
361	GGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121	G T K L T V L G Q P K A A P S V T L F P
421	CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141	P S S E E L Q A N K A T L V C L I S D F
481	TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161	Y P G A V T V A W K A D S S P V K A G V
541	GAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGCCAGCAGCTACCTGAGC
181	E T T T P S K Q S N N K Y A A S S Y L S
601	CTGACGCCTGAGCAGTGGAGTCCCAAAAAGCTACAGCTGCCAGGTCACGCATGAAGGG
201	L T P E Q W K S H K S Y S C Q V T H E G
661	AGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATGA
221	S T V E K T V A P T E C S *

# FIG. 49

	1	11	21	31	41	51	
1	EVOLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA	ISGSGGSTYY	60
61	ADSVKGRFTI	SRDNSKNTLY	LOMNSLRAED	TAVYYCARVA	KTLMROYSLW	GQGTTLVTISR	120
121	GGGSGGGGGS	GGGGSELTQ	DPAVSVALGQ	TVRITCQGDS	LRSYVASWYQ	QKPGQAPVLV	180
181	IYGKNNRPSG	IPDRFSGSSS	GNTASLTITG	AQAEDEADYY	CNSRDSSGNH	VVFGGGTKLT	240
241	VLGAAAEQKL	ISEEDLNGAA					

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# FIG. 50

		10	20	30	40	50	60
1	3	AtTATTACTc gCGGCCcAGC CgGCCcAGC CGAGGTGCAG CTGGTGGAGT CTGGGGGAGG					
		L L L A A Q P A M A E V Q L V E S G G G					
		70	80	90	100	110	120
1	3	CTTGGTACAG CCTGGGGGGT CCTGAGACT CTCCTGTGCA GCCTCTGGAT TCACCTTTAG					
		L V Q P G G S L R L S C A A S G F T F S					
		130	140	150	160	170	180
1	3	CAGCTATGCC ATGAGCTGGG TCCGCCAGGC TCCAGGGAAG GGCCTGGAGT GGGTCTCAGC					
		S Y A M S W V R Q A P G K G L E W V S A					
		190	200	210	220	230	240
1	3	TATTAGTGGT AGTGGTGGTA GCACATACTA CGCAGACTCC GTGAAGGGCC GGTTCACCAT					
		I S G S G G S T Y Y A D S V K G R F T I					
		250	260	270	280	290	300
1	3	CTCCAGAGAC AATTCCAAGA ACACGCTGTA TCTGCAAATG AACAGCCTGA GAGCCGAGGA					
		S R D N S K N T L Y L Q M N S L R A E D					
		310	320	330	340	350	360
1	3	CACGGCCGTG TATTACTGTG CAAGACCGG CACAGATATT AAGCGTAAAT GGGGCCAAGG					
		T A V Y Y C A R T G Q S I K R S W G Q G					
		370	380	390	400	410	420
1	3	TACCCTGGTC ACCGTGTGCA GAGGTGGAGG CGGTTCAGGC GGAGGTGgCT CTGGCGGTGG					
		T L V T V S R G G G G S G G G G S G G G					
		430	440	450	460	470	480
1	3	CGGATCGTCT GAgCTGACTC AGGACCCTGC TGTGTCTGTG GcCTTGGGAC AgACAGTCAG					
		G S S E L T Q D P A V S V A L G Q T V R					
		490	500	510	520	530	540
1	3	GATcACATGC CAAGGAgACA GCCTCAGAAG CTATTATGCA AGCTGGTACC AGCAGAAGCC					
		I T C Q G D S L R S Y Y A S W Y Q Q K P					
		550	560	570	580	590	600
1	3	AGGACAGGCC CCTGTACTTG TCATCTATGG TAAAAACAAC CGGCCCTCAG GGATCCCAGA					
		G Q A P V L V I Y G K N N R P S G I P D					
		610	620	630	640	650	660
1	3	CCGATTCTCT GGCTCCAGCT CAGGAAACAC AGCTTCCTTG ACCATCACTG GGGCTCAGGC					
		R F S G S S S G N T A S L T I T G A Q A					
		670	680	690	700	710	720
1	3	GGAAGATGAG GCTGACTATT ACTGTAACTC CCGGGACAGC AGTGGTAACC ATGTGGTATT					
		E D E A D Y Y C N S R D S S G N H V V F					
		730	740	750	760	770	780
1	3	CGGCGGAGGG ACCAAGCTGA CGTCCTAGG TGCGGCCGCA GAACAAAAC TCATCTCAGA					
		G G G T K L T V L G A A A E Q K L I S E					
		790	800	810	820	830	840
1	3	AGAgGAtCTG AatGGGGCCG CAAGAACTG TtGAATTTT TAAGTTAAc T					
		E D L N G A A * N C * I F * V N					

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Y/16 SEQ ID NO: 210

## ***FIG. 51***

Sequence of Y1-Biotag (SEQ ID NO: 211)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ  
41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDN AKNSL  
81 YLQMNSLRAE DTA VYYCARM RAPVIWGQGT LVT VSRGGGG  
121 SGGGGSGGGG SSEL TQDPAV SVALGQTVRI TCQGDSLRSY  
161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA  
201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG  
241 GGLNDIFEAQ KIEWHE

## ***FIG. 52***

Y1-cys-kak scFv (SEQ ID NO. 212)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ  
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM  
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY  
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR  
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK

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